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(54) Title: ANTI-INFLAMMATORY COMPOUNDS			
(57) Abstract			
<p>This invention relates to the novel compounds and pharmaceutical compositions of formula (I) and of formula (II). This invention also relates to a method of treating or reducing inflammation in a mammal in need thereof, which comprises administering to said mammal an effective amount of a compound or composition of formula (I) or (II).</p>			

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## ANTI-INFLAMMATORY COMPOUNDS

## FIELD OF THE INVENTION

10 This invention relates to pharmaceutical compositions and their use as anti-inflammatory agents in mammals.

## BACKGROUND OF THE INVENTION

15 An early event in the response of most inflammatory cells to immunologic activation and other stimuli is the release of newly formed products (mediators) which alter the function and biochemistry of surrounding cells and tissues. The ensuing biological responses, as well as much of the pathogenesis which is attributed to inflammation and 20 allergy, are thought to be dependent on the effects that these newly-formed mediators have on adjacent cells within the inflammatory region.

25 In the last 20 years, it has become apparent that lipid mediators are among the most potent and important products which are generated during inflammatory reactions. The synthesis of most lipid mediators is initiated by the specific cleavage of complex phospholipid molecules which contain arachidonate at their sn-2 position. Arachidonic acid is predominantly found in the sn-2 position of phospholipids after redistribution by transacylases and its release by sn-2 acylhydrolases from phospholipids represents the rate-limiting step in the formation of eicosanoids (leukotrienes, prostaglandins and thromboxanes) and other hydroxylated fatty acids. As arachidonic acid is released, it is then converted to oxygenated derivatives by at least two enzymatic systems (lipoxygenase and/or cyclooxygenase). Concomitant with arachidonate release, lysophospholipids are formed. One of these lyso phospholipids, 1-alkyl-2-lyso-sn-glycero-3-phosphocholine, is then 30 acetylated to form platelet-activating factor (PAF). Each of the cell types involved in the inflammatory response produce and secrete a unique subset of lipid mediators. The quantities and nature of the metabolites depend on which enzymes and precursor phospholipid pools are available to inflammatory cells.

35 Once lipid mediators such as PAF and eicosanoids are formed by the aforementioned pathways, they induce signs and symptoms observed in the pathogenesis of various inflammatory disorders. Indeed, the pathophysiological activity of arachidonic acid (and its metabolites) is well known to those skilled in the art. For example, these mediators have been implicated as having an important role in allergy, asthma, anaphylaxis, adult

respiratory distress syndrome, reperfusion injury, inflammatory bowel disease, rheumatoid arthritis, endotoxic shock, and cardiovascular disease. Aalmon et al., Br. Med. Bull (1978) 43:285-296; Piper et al., Ann. NY Acad. Sci. (1991) 629:112-119; Holtzman, Am. Rev. Respir. Dis. (1991) 143:188-203; Snyder, Am. J. Physiol. Cell Physiol. (1990) 259:C697-5 C708; Prescott et al., J. Biol. Chem. (1990) 265:17381-17384.

Similar to arachidonate products, PAF is a potent proinflammatory mediator produced by a variety of cells. *In vitro*, PAF stimulates the movement and aggregation of neutrophils and the release therefrom of tissue-damaging enzymes and oxygen radicals. PAF has also been implicated in activation of leukocytes, monocytes, and macrophages. 10 These activities contribute to the actions of PAF as having (pathological) physiological activity in inflammatory and allergic responses. PAF has also been implicated in smooth muscle contraction, pain, edema, hypotensive action, increases in vascular permeability, cardiovascular disorders, asthma, lung edema, endotoxin shock, and adult respiratory distress syndrome. PAF elicits these responses either directly through its own cellular 15 receptor(s) or indirectly by inducing the synthesis of other mediators.

Accordingly, a method which antagonises the production of free arachidonic acid, its metabolites or PAF will have clinical utility in the treatment of a variety of allergic, inflammatory and hypersecretory conditions such as asthma, arthritis, rhinitis, bronchitis and urticaria, as well as reperfusion injury and other disease involving lipid mediators of 20 inflammation. Many published patent applications or issued US patents exist which describe various compounds having utility as PAF or eicosanoid antagonists. Such patents include U.S. Pat. No. 4,788,205, 4,801,598, 4,981,860, 4,992,455, 4,983,592, 5,011,847, 5,019,581 and 5,002,941.

25 Phospholipase A2's (PLA<sub>2</sub> (EC 3.1.1.4)) are responsible for the liberation of arachidonic acid from the sn-2 position of phospholipid. They are thought to play an important role in the pathogenesis of inflammation and possibly in immunological dysfunction, both as a cell associated enzyme as well as an extracellular soluble enzyme. Low molecular weight, mammalian Type II 14 kDa PLA<sub>2</sub> has been well characterized and is known to exist in both an extracellular form in inflammatory fluids (Kramer et al., J. Biol. 30 Chem., 264:5768-5775 (1989) and in a cell associated form (Kanda et al., Biochemical and Biophysical Research Communications, 163:42-48 (1989) and has been found in a variety of cells and tissues or extracellularly when released in response to antigenic activators or pro-inflammatory mediators such as Interleukin (IL)-1, IL-6 or tumor necrosis factor (TNF). Its presence in such inflammatory fluids, tissue exudates or serum has therefore 35 implicated Type II-14 kDa-PLA<sub>2</sub>'s role in inflammation (Vadas, et al., (1985) Life Sci. 36, 579-587; and Seilhamer, et al., (1989) J. Biol. Chem. 264, 5335-5338). Recently, the elevated serum levels of PLA<sub>2</sub> activity during an inflammatory insult has been attributed to

cytokine induction of acute phase protein release from liver, of which the 14 kDa-PLA<sub>2</sub> is suggested to be a part (Crowl, et al., (1991) *J. Biol. Chem.* 266, 2647-2651). In addition, soluble PLA<sub>2</sub> activity is markedly elevated in the serum and synovial fluid of patients with rheumatoid arthritis (Stefanski et al., *J. Biochem.* 100:1297-303 (1986)).

5 Furthermore, increasing serum PLA<sub>2</sub> levels have been shown to positively correlate with clinical severity (Bomalaski and Clark, *Arthritis and Rheumat.* 36:190-198 (1993)). Various inhibitors of PLA<sub>2</sub> have been described in publications and in US Patents. See for instance US Patents 4,959,357; 4,933,365; 5,208,223; 5,208244; Marshall et al., *J. Rheumatology* 18:1 (1991); Marshall et al., *Phospholipase A2*, Ed. Pyu Wong, Plenum Press, NY (1990) pages 169-181; Wilkerson, et al., *Eur. J. Med. Chem.*, 26:667, 1991 and Wilkerson, *Antiinflammatory Phospholipase A2 Inhibitors, Drugs of the Future*, Vol. 15, No. 2 p 139-148(1990). Accordingly, as PLA<sub>2</sub> is important in the liberation of arachidonic acid from phospholipid and may also play a role in the generation of PAF via lysophospholipid formation, inhibition of such an enzyme would be useful for the treatment

10 15 of disease states caused thereby.

There are many novel forms of phospholipase A2's which have recently been discovered. For the purposes herein, members of the sn-2 acylhydrolase family of PLA2's are divided into low and high molecular weight enzymes be it from mammalian, or non-mammalian sources. Low molecular weight PLA2's will generally have a molecular weight

20 25 in the range of 12,000 to 15,000. High molecular weight will be in the range of 30,000 or 56,000 kDa to 110,000 by SDS electrophoresis analysis.

A high molecular weight, cytosolic 85 kDa PLA<sub>2</sub> has been isolated and cloned from the human moncytic cell line, U937 (Clark et al., *Proc. Natl. Acad. Sci.*, 87:7708-7712, 1990). The cell-associated Type II-14 kDa-PLA<sub>2</sub> in cell lipid metabolism was thought to be the key rate limiting enzyme in lipid mediator formation, until the recent identification of this cell-associated but structurally distinct 85 kDa sn-2 acylhydrolase, (Clark, et al., *supra*); and Kramer, et al., (1991) *J. Biol. Chem.* 266, 5268-5272. Like the Type II-14 kDa enyzme, this enzyme is active at neutral pH and Ca<sup>2+</sup>-dependent, but in contrast exhibits a preference for AA in the sn-2 position of phospholipid substrate and migrates from the cytosol to the membrane in a Ca<sup>2+</sup>-dependent manner and is regulated by phosphorylation (Kramer et al., *J. Biol. Chem.*, 266:5268-5272 (1991)). The 85 kDa-PLA<sub>2</sub> is also distinct from 14 kDa-PLA<sub>2</sub>s and Ca<sup>2+</sup>-independent PLA<sub>2</sub> as demonstrated by different biochemical characteristics such as stability of the 85 kDa-PLA<sub>2</sub> to DTT, instability to heat and the lack of inhibition by a phosphonate phospholipid TSA inhibitor of 14 kDa-PLA<sub>2</sub>. In addition, 85 kDa-PLA<sub>2</sub> has been shown to possess a lysophospholipase A<sub>1</sub> activity which is not observed with the 14 kDa-PLA<sub>2</sub>s. The 85 kDa enzyme is similar to the myocardial Ca<sup>2+</sup>-independent PLA<sub>2</sub> (Bomalaski and Clark, *Arthritis and Rheumat.* 36:190-198 (1993)) in that Ca<sup>2+</sup> is not

required for catalysis and DTNB inhibition is observed. However, 85 kDa-PLA<sub>2</sub> is not inhibited by the suicide inactivator bromoenol lactone, suggesting that the enzyme is distinct from the myocardial enzyme as well.

These characteristics make the 85 kDa-PLA<sub>2</sub> a candidate for participation in the 5 liberation of AA from phospholipid stores for subsequent metabolism to lipid mediators. Both the cytosolic 85 kDa PLA<sub>2</sub> and a cell associated Type II 14 kDa PLA<sub>2</sub> have been found in the human monocyte, neutrophil and platelet (Marshall and Roshak, *Biochem. Cell Biol.* 71:331-339 (1993)). As noted above most of the cellular lipid mediators found elevated in a variety of inflammatory fluids are formed in response to non-pancreatic 14 kDa 10 PLA<sub>2</sub> action. Since arachidonate-containing phospholipids are the key precursors for a broad range of lipid mediators it would not be surprising that, inflammatory cells would treat these phospholipids differently than other fatty acid-containing phospholipids. In particular, there are enzymes which control the amount of arachidonate in different phospholipid pools and these enzymes are tightly regulated to maintain arachidonate 15 homeostasis. The movement of arachidonate into and from all phospholipids was originally thought to be exclusively by Coenzyme A-dependent acyl transferase activities. Holub *et al.*, *Adv. Lipid Res.*, 16:1-125 (1978); Lands *et al.*, In *The Enzymes of Biological Membranes*, ed. Martonosi, A., pp. 3-85, Plenum Press, NY, 1976. However, it has now been demonstrated that an enzyme, Coenzyme A-independent transacylase (CoA-IT), is 20 involved in the movement of 20 carbon higher unsaturated fatty acids, particularly arachidonate, into particular (1-alkyl- and 1-alkenyl) phospholipid pools. These are the phospholipid pools of arachidonate that are preferentially mobilized during cell activation and utilized for eicosanoid and PAF biosynthesis, respectively.

CoA-IT has a specificity for certain phospholipids as donor and acceptor molecules. 25 The fatty acid transferred is long chained and unsaturated, and almost exclusively arachidonate. Other fatty acids such as the 16:0, 18:1 or 18:2 are not moved into the sn-2 position of alkyl and 1-alkenyl phospholipid pools by CoA-IT. The specificity of CoA-IT is in direct contrast to many other CoA-dependent acylation activities which acylate a wide variety of lysophospholipids with no selectivity for arachidonate.

30 Accordingly, as CoA-IT is involved in arachidonic acid and phospholipid metabolism, inhibition of such an enzyme would be useful for the treatment of inflammatory, allergic and hypersecretory conditions or disease states caused thereby. Therefore, a method by which CoA-IT is inhibited will consequently and preferentially decrease the arachidonate content of 1-alkyl- and 1-alkenyl-linked phospholipids and will 35 therefore decrease the production of pro-inflammatory mediators such as free arachidonic acid, prostaglandins, leukotriene and PAF during an inflammatory response.

## SUMMARY OF THE INVENTION

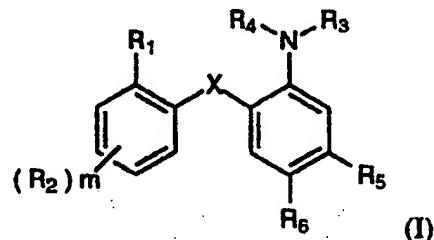
This invention relates to the novel compounds of Formula (I) and pharmaceutical compositions thereof which comprises a compound of Formula (I), or pharmaceutically acceptable salt thereof and a pharmaceutically acceptable diluent or carrier.

5 This invention also relates to a method of treating or reducing inflammation in a mammal in need thereof, which comprises administering to said mammal an effective amount of a compound or composition of Formula (I).

10 This invention also relates to a method of treating disease or disorders mediated by lipid inflammatory mediators, free arachidonic acid, its metabolites and/or PAF by administering to a patient in need thereof, an effective amount of a compound of Formula (I).

This invention also relates to a method of treating disease or disorders mediated by phospholipase A2 (PLA2) and/or Coenzyme A-independent transacylase (CoA-IT) by administering to a patient in need thereof, an effective amount of a compound or composition of Formula (I).

15 One aspect of the present invention are compounds having the structure represented by the formula:



wherein

20  $R_1$  is  $SO_3H$  or  $S(O)_nC_{1-4}$  alkyl;  
 $n$  is 0 or an integer having a value of 1 or 2;  
 $R_2$  is hydrogen, halogen, optionally substituted  $C_{1-8}$  alkyl, or  $C_{1-8}$  alkoxy;  
 $m$  is an integer having a value of 1 or 2;  
 $R_3$  is  $-C(O)R_7$  or  $-C(S)R_7$ ;

25  $R_4$  is hydrogen, or  $C_{1-4}$  alkyl;  
 $R_5$  is hydrogen, halogen,  $CF_3$ ,  $CH_3$ ,  $(CH_2)_tC(O)R_8$ , or  $(CH_2)_tOH$ ;  
 $t$  is 0 or an integer having a value of 1 or 2;  
 $R_6$  is hydrogen or halogen;

30  $R_7$  is  $NR_9R_{10}$ ;  
 $R_8$  is hydrogen or  $C_{1-4}$  alkyl;  
 $R_9$  is hydrogen or  $C_{1-4}$  alkyl;  
 $R_{10}$  is optionally substituted aryl, optionally substituted aryl $C_{1-2}$  alkyl, optionally

substituted C<sub>1</sub>-8 alkyl, or R<sub>9</sub> and R<sub>10</sub> together with the nitrogen to which they are attached form a 5 to 7 membered saturated or unsaturated ring which may optionally comprise an additional heteroatom selected from O/N or sulfur;

X is oxygen or sulfur;

5 provided that

- a) when R<sub>2</sub> is 2,2-dimethyl propyl, m is 1, R<sub>1</sub> is SO<sub>3</sub>H, R<sub>5</sub> is CF<sub>3</sub>, and R<sub>3</sub> is C(O)NR<sub>9</sub>R<sub>10</sub>, and R<sub>9</sub> is hydrogen, than R<sub>10</sub> is other than 3,5-trimethyl phenyl; or
- b) when R<sub>2</sub> is 2,2-dimethyl propyl, m is 1, R<sub>1</sub> is SO<sub>3</sub>H, R<sub>5</sub> is CF<sub>3</sub>, and R<sub>3</sub> is C(O)NR<sub>9</sub>R<sub>10</sub> and R<sub>9</sub> is hydrogen, than R<sub>10</sub> is other than 4-chloro-3-trifluoromethyl phenyl;
- 10 c) when R<sub>2</sub> is 2,2-dimethyl propyl, m is 1, R<sub>1</sub> is SO<sub>3</sub>H, R<sub>5</sub> is CF<sub>3</sub>, and R<sub>3</sub> is C(O)NR<sub>9</sub>R<sub>10</sub> and R<sub>9</sub> is hydrogen, than R<sub>10</sub> is other than 3-trifluoromethyl phenyl;
- d) when R<sub>1</sub> is SO<sub>3</sub>H, R<sub>2</sub> is 5-methyl-4-chloro, m is 2, R<sub>5</sub> is chlorine, R<sub>6</sub> is hydrogen, and R<sub>3</sub> is C(O)NR<sub>9</sub>R<sub>10</sub> and R<sub>9</sub> is hydrogen, than R<sub>10</sub> is other than a 3-CF<sub>3</sub>-4-chlorophenyl, 3,4-dichlorophenyl, or 2-methyl-6-chlorophenyl;
- 15 e) when R<sub>1</sub> is SO<sub>3</sub>H, R<sub>2</sub> is 4,6-dichloro, m is 2, R<sub>5</sub> is chlorine, R<sub>6</sub> is hydrogen, and R<sub>3</sub> is C(O)NR<sub>9</sub>R<sub>10</sub> and R<sub>9</sub> is hydrogen, than R<sub>10</sub> is other than a 2-methyl-4-chlorophenyl;
- f) when R<sub>1</sub> is SO<sub>3</sub>H, R<sub>2</sub> is 4-chloro, m is 1, R<sub>5</sub> is chlorine, R<sub>6</sub> is hydrogen, and R<sub>3</sub> is C(O)NR<sub>9</sub>R<sub>10</sub> and R<sub>9</sub> is hydrogen, than R<sub>10</sub> is other than a 2,5-dichlorophenyl, 3,5-di-15 trifluoromethyl phenyl, 2-chloro-5-CF<sub>3</sub>-phenyl, 3-CF<sub>3</sub>-4-chlorophenyl, 3,4-dichlorophenyl, 2,3-dichlorophenyl, 4-chlorophenyl, 3-trifluoromethylphenyl, 3-chloro-4-(4-chlorophenoxy), 3-CF<sub>3</sub>-6-(4-chlorophenoxy), 3-chloro-6-(4-chlorophenoxy);
- 20 g) when R<sub>1</sub> is SO<sub>3</sub>H, R<sub>2</sub> is 4,5-dichloro, m is 2, R<sub>5</sub> is chlorine, R<sub>6</sub> is hydrogen, and R<sub>3</sub> is C(O)NR<sub>9</sub>R<sub>10</sub> and R<sub>9</sub> is hydrogen, than R<sub>10</sub> is other than a 2-chloro-5-CF<sub>3</sub>-phenyl or 3-CF<sub>3</sub>-4-chlorophenyl;
- 25 h) when R<sub>1</sub> is SO<sub>3</sub>H, R<sub>2</sub> is 4,5-dichloro, m is 2, R<sub>5</sub> is CF<sub>3</sub>, R<sub>6</sub> is hydrogen, and R<sub>3</sub> is C(O)NR<sub>9</sub>R<sub>10</sub> and R<sub>9</sub> is hydrogen, than R<sub>10</sub> is other than a 4-chloro-3-CF<sub>3</sub>phenyl;
- 30 i) when R<sub>1</sub> is SO<sub>3</sub>H, X is sulfur, R<sub>2</sub> is 4,5-dichloro, m is 2, R<sub>5</sub> is trifluoromethyl, R<sub>6</sub> is hydrogen, and R<sub>3</sub> is C(O)NR<sub>9</sub>R<sub>10</sub> and R<sub>9</sub> is hydrogen, than R<sub>10</sub> is other than a phenyl substituted one to two times independently with chlorine or trifluoromethyl;
- j) when R<sub>1</sub> is SO<sub>3</sub>H, X is sulfur, R<sub>2</sub> is 4-chloro, m is 1, R<sub>5</sub> is trifluoromethyl, R<sub>6</sub> is hydrogen, and R<sub>3</sub> is C(O)NR<sub>9</sub>R<sub>10</sub> and R<sub>9</sub> is hydrogen, than R<sub>10</sub> is other than a phenyl substituted one to two times independently with chlorine or trifluoromethyl;
- 35 k) when R<sub>1</sub> is SO<sub>3</sub>H, X is sulfur, R<sub>2</sub> is 4-chloro, m is 1, R<sub>5</sub> is chlorine, R<sub>6</sub> is hydrogen, and R<sub>3</sub> is C(O)NR<sub>9</sub>R<sub>10</sub> and R<sub>9</sub> is hydrogen, than R<sub>10</sub> is other than a phenyl

substituted one to two times independently with chlorine or trifluoromethyl; or a pharmaceutically acceptable salt thereof.

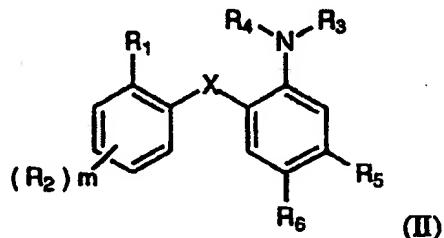
5 This invention also relates to the novel compounds of Formula (II) and pharmaceutical compositions thereof which comprises a compound of Formula (II), or pharmaceutically acceptable salt thereof and a pharmaceutically acceptable diluent or carrier.

This invention also relates to a method of treating or reducing inflammation in a mammal in need thereof, which comprises administering to said mammal an effective amount of a compound or composition of Formula (II).

10 This invention also relates to a method of treating disease or disorders mediated by free arachidonic acid, its metabolites and/or PAF by administering to a patient in need thereof, an effective amount of a compound of Formula (II).

This invention also relates to a method of treating disease or disorders mediated by phospholipase A2 (PLA2) and/or Coenzyme A-independent transacylase (CoA-IT) by 15 administering to a patient in need thereof, an effective amount of a compound or composition of Formula (II).

One aspect of the present invention are compounds having the structure represented by the formula:



wherein

R<sub>1</sub> is SO<sub>3</sub>H or S(O)<sub>n</sub>C<sub>1-4</sub> alkyl;

n is 0 or an integer having a value of 1 or 2;

25 R<sub>2</sub> is hydrogen, halogen, optionally substituted C<sub>1-8</sub> alkyl, or C<sub>1-8</sub> alkoxy;

m is an integer having a value of 1 or 2;

R<sub>3</sub> is -C(O)R<sub>7</sub> or -C(S)R<sub>7</sub>;

R<sub>4</sub> is hydrogen, or C<sub>1-4</sub> alkyl;

R<sub>5</sub> is hydrogen, halogen, CF<sub>3</sub>, CH<sub>3</sub>, (CH<sub>2</sub>)<sub>t</sub>C(O)R<sub>8</sub>, or (CH<sub>2</sub>)<sub>t</sub>OH;

30 t is 0 or an integer having a value of 1 or 2;

R<sub>6</sub> is hydrogen or halogen;

R<sub>7</sub> is optionally substituted aryl, optionally substituted arylC<sub>1-2</sub> alkyl, or optionally

substituted C<sub>1</sub>-8 alkyl ;  
R<sub>8</sub> is hydrogen or C<sub>1</sub>-4 alkyl;  
X is oxygen or sulfur;  
or a pharmaceutically acceptable salt thereof.

5

#### DETAILED DESCRIPTION OF THE INVENTION

The present invention is directed to a novel method of treating inflammatory disease in a mammal in need thereof by administering to said mammal an effective amount of a compound according to Formula (I) or (II). The compounds of Formula (I) and (II) may 10 selectively inhibit the PLA<sub>2</sub> enzyme, the CoA-IT enzyme or both. Inhibition of either or both enzymes will result in the treatment of inflammatory occurrences in mammals. Inflammatory states in mammals may include, but are not limited to, allergic and asthmatic manifestations, dermatological diseases, inflammatory diseases, collagen diseases, reperfusion injury and stroke. Treatment of both acute and chronic diseases are possible.

15 Preferred diseases for treatment are arthritis, asthma, allergic rhinitis, inflammatory bowel disease (IBD), psoriasis, reperfusion injury and stroke. For the purposes herein, the compounds of Formula (I) and (II) are preferential and selective inhibitors of the low molecular weight PLA<sub>2</sub> enzyme.

20 For compounds of Formula (I) R<sub>1</sub> is suitably SO<sub>3</sub>H or S(O)<sub>n</sub>C<sub>1</sub>-4 alkyl, and n is a number having a value of 0 to 2. Preferably R<sub>1</sub> is SO<sub>3</sub>H or a salt thereof.

Suitably, R<sub>2</sub> is independently a substituent on the benzene ring from 0 to 2 times, and such substituent is suitably selected from hydrogen, halogen, an optionally substituted C<sub>1</sub>-8 alkyl, or C<sub>1</sub>-8 alkoxy group. Suitably when R<sub>2</sub> is halogen it is a chlorine or bromine.

25 When R<sub>2</sub> is an optionally substituted C<sub>1</sub>-8 alkyl, the alkyl is substituted one to three times with halogen, such as fluorine, preferably a trifluoromethyl group. The optionally substituted C<sub>1</sub>-8 alkyl moiety is preferably a branched C<sub>5</sub> chain, such as 1,1-dimethyl propyl moiety or a C<sub>8</sub> branched chain such as 1,1,3,3-tetramethyl butyl moiety. Suitably m is an integer having a value of 1 or 2. Most preferably R<sub>2</sub> is hydrogen.

30 Suitably, R<sub>3</sub> is C(O)R<sub>7</sub> or -C(S)R<sub>7</sub> and R<sub>7</sub> is suitably optionally substituted aryl, an optionally substituted arylC<sub>1</sub>-2 alkyl, an optionally substituted C<sub>1</sub>-8 alkyl group or the group NR<sub>9</sub>R<sub>10</sub>. Suitably R<sub>9</sub> is hydrogen or C<sub>1</sub>-4 alkyl; and R<sub>10</sub> is hydrogen, optionally substituted aryl, optionally substituted arylC<sub>1</sub>-2 alkyl, optionally substituted C<sub>1</sub>-8 alkyl, or together R<sub>9</sub> and R<sub>10</sub> with the nitrogen to which they are attached form a 5 to 7 membered 35 saturated or unsaturated ring which may optionally comprise an additional heteroatom selected from O/N or sulfur.

Suitably the aryl, aryl alkyl or alkyl moieties of R<sub>7</sub> and R<sub>9</sub> and R<sub>10</sub> are substituted

independently, one to three times, by halogen, trifluoromethyl, aryloxy, methoxy,  $\text{CH}_2\text{OH}$ , methyl, or  $\text{C}(\text{O})_2\text{H}$ . Preferably, the substituents are halogen, or trifluoromethyl. The substituent halogen groups are preferably Cl, Br and fluorine. More preferably the substituents are in the 3,5- position or the 4-position of the aryl ring. Suitably the substituents are 3,5-bis-trifluoromethyl, 4-trifluoromethyl, 4-bromo, 4-chloro, or 4-fluoro.

When  $\text{R}_7$  is an aryl moiety it is preferably phenyl; when  $\text{R}_7$  is an arylalkyl moiety it is preferably benzyl; when  $\text{R}_7$  is an alkyl moiety it is preferably a methyl or a  $\text{C}_8$  unbranched chain. The methyl moiety if substituted is preferably substituted by fluorine to yield a trifluoromethyl group. More preferably  $\text{R}_7$  is an optionally substituted phenyl.

10 Suitably the aryl substituents are 3,5-bis-trifluoromethyl, 4-trifluoromethyl, 4-bromo, 4-chloro, or 4-fluoro.

When  $\text{R}_7$  is  $\text{NR}_9\text{R}_{10}$  the  $\text{R}_9$  group is preferably hydrogen. When  $\text{R}_{10}$  is an aryl moiety it is preferably phenyl; when  $\text{R}_{10}$  is an aryl alkyl moiety it is preferably benzyl; when  $\text{R}_{10}$  is an alkyl moiety it is preferably a methyl or a  $\text{C}_8$  unbranched chain. The methyl moiety, if substituted, is preferably substituted by fluorine to yield a trifluoromethyl group. More preferably  $\text{R}_{10}$  is an optionally substituted phenyl as defined above.

Suitably  $\text{X}$  is oxygen or sulfur, preferably oxygen.

Suitably  $\text{R}_4$  is hydrogen, or  $\text{C}_{1-4}$  alkyl, preferably hydrogen.

20 Suitably  $\text{R}_5$  is hydrogen, halogen,  $\text{CF}_3$ ,  $(\text{CH}_2)_t\text{C}(\text{O})_2\text{R}_8$ , or  $(\text{CH}_2)_t\text{OH}$ , and  $t$  is 15 or an integer having a value of 1 or 2. Preferably  $t$  is 1. Preferably when  $\text{R}_5$  is  $(\text{CH}_2)_t\text{C}(\text{O})_2\text{R}_8$ ,  $\text{R}_8$  is a  $\text{C}_{1-4}$  alkyl group, preferably t-butyl. Preferred  $\text{R}_5$  groups are hydrogen,  $\text{CF}_3$ , or halogen. More preferably  $\text{R}_5$  is hydrogen or  $\text{CF}_3$ .

Suitably  $\text{R}_6$  is hydrogen or halogen; preferably hydrogen. If  $\text{R}_6$  is halogen it is preferably fluorine or chlorine.

25

Specifically exemplified compounds of Formula (I) are:

2-[2-(3-Phenylureido)-4-trifluoromethylphenoxy]-5-(1,1-dimethylpropyl)benzenesulfonic acid;

2-[2-(3-Phenylureido)phenoxy]-5-(1,1-dimethylpropyl)benzenesulfonic acid; 2-[2-[3-[3,5-

30 Bis(trifluoromethyl)phenyl]ureido]phenoxy]-5-(1,1-dimethylpropyl)benzenesulfonic acid; 2-[2-[3-(4-Phenoxyphenyl)ureido]-4-trifluoromethylphenoxy]-5-(1,1-dimethylpropyl)-benzenesulfonic acid;

2-[2-[3-(4-Methoxyphenyl)ureido]-4-trifluoromethylphenoxy]-5-(1,1-dimethylpropyl)-benzenesulfonic acid;

35 2-[2-[3-[3,5-Bis(trifluoromethyl)phenyl]ureido]-4-trifluoromethylphenoxy]-5-(1,1,3,3-tetramethylbutyl)benzenesulfonic acid;

2-[2-[3-[3,5-bis(Trifluoromethyl)phenyl]ureido]-4-methylphenoxy]-5-(1,1-

dimethylpropyl)benzenesulfonic acid;  
2-[2-[3-[3,5-bis(trifluoromethyl)phenyl]thioureido]-4-(trifluoromethyl)phenoxy]-5-(1,1-dimethylpropyl)benzenesulfonic acid; and  
2-[2-[3-[3,5-Bis(trifluoromethyl)phenyl]ureido]-4-trifluoromethyl)phenoxy]-5-benzenesulfonic acid.

For compounds of Formula (II) the variable R<sub>1</sub>, R<sub>2</sub>, m, t, R<sub>4</sub>, R<sub>5</sub>, R<sub>6</sub>, and R<sub>8</sub> are as preferably as indicated above for compounds of Formula (I).

Suitably, for compounds of Formula (II), R<sub>3</sub> is -C(O)R<sub>7</sub> or -C(S)R<sub>7</sub> and R<sub>7</sub> is an 10 optionally substituted aryl, an optionally substituted arylC<sub>1-2</sub> alkyl, or an optionally substituted C<sub>1-8</sub> alkyl group. Preferably R<sub>3</sub> is -C(O)R<sub>7</sub>.

Suitably the aryl, arylalkyl or alkyl moieties of R<sub>7</sub> are substituted independently, one to three times, by halogen, trifluoromethyl, aryloxy, methoxy, CH<sub>2</sub>OH, methyl, or C(O)H. Preferably, the substituents are halogen, or trifluoromethyl. The substituent halogen groups 15 are preferably Cl, Br and fluorine. More preferably the substituents are in the 3,5- position or the 4-position of the aryl ring. Suitably the substituents are 3,5-bis-trifluoromethyl, 4-trifluoromethyl, 4-bromo, 4-chloro, or 4-fluoro.

When R<sub>7</sub> is an aryl moiety it is preferably phenyl; when R<sub>7</sub> is an aryl alkyl moiety it is preferably benzyl; when R<sub>7</sub> is an alkyl moiety it is preferably a methyl or a C<sub>8</sub> 20 unbranched chain. The methyl moiety if substituted is preferably substituted by fluorine to yield a trifluoromethyl group. More preferably R<sub>7</sub> is an optionally substituted phenyl or benzyl group.

Specifically exemplified compounds of Formula (II) include:

25 2-[2-(Phenylmethylacetamido)phenoxy]-5-(1,1-dimethylpropyl)benzenesulfonic acid, and its sodium salt  
2-[2-(Benzamido)-4-trifluoromethylphenoxy]-5-(1,1-dimethylpropyl)benzenesulfonic acid, sodium salt  
2-[2-[3,5-bis(trifluoromethyl)benzamido-4-trifluoromethylphenoxy]-5-(1,1-30 dimethylpropyl)benzenesulfonic acid, and its sodium salt

Suitable pharmaceutically acceptable salts are well known to those skilled in the art and include basic salts of inorganic and organic acids, such as hydrochloric acid, hydrobromic acid, sulphuric acid, phosphoric acid, methane sulphonic acid, ethane 35 sulphonic acid, acetic acid, malic acid, tartaric acid, citric acid, lactic acid, oxalic acid, succinic acid, fumaric acid, maleic acid, benzoic acid, salicylic acid, phenylacetic acid and mandelic acid. In addition, pharmaceutically acceptable salts of compounds of formula (I)

may also be formed with a pharmaceutically acceptable cation, if possible. Suitable pharmaceutically acceptable cations are well known to those skilled in the art and include alkaline, alkaline earth, ammonium and quaternary ammonium cations.

For purposes herein, the compounds of the generic formulas (I) and (II), for the (R<sub>2</sub>)<sub>m</sub> term provisos, etc. are numbered by the point of attachment to the ether or thioether. This point of attachment is the one position, the R<sub>1</sub> term is the 2-position, etc. The nomenclature used for naming the specifically exemplified compounds, such as common or IUPAC corresponds to actual rules of nomenclature as is unaffected by the generic formula herein.

10 The following terms, as used herein, refer to:

- "halo" - all halogens, that is chloro, fluoro, bromo and iodo;
- "C<sub>1-8</sub> alkyl" or "alkyl" - both straight and branched chain radicals of 1 to 8 carbon atoms, unless the chain length is otherwise limited, including, but not limited to, methyl, ethyl, *n*-propyl, *iso*-propyl, *n*-butyl, *sec*-butyl, *iso*-butyl, *tert*-butyl, and the like;

15 • "aryl" - phenyl and naphthyl;

• "aryl alkyl" or "heteroaryl alkyl" or "heterocyclic alkyl" - is used herein to mean a aryl, heteroaryl or heterocyclic ring connected to a C<sub>1-4</sub> alkyl as defined above unless otherwise indicated;

• "heteroaryl" (on its own or in any combination, such as "heteroaryloxy") - a 5-10

20 membered aromatic ring system in which one or more rings contain one or more heteroatoms selected from the group consisting of N, O or S, such as, but not limited, to pyrrole, quinoline, isoquinoline, pyridine, pyrimidine, oxazole, thiazole, thiadiazole, triazole, imidazole, or benzimidazole;

• "heterocyclic" (on its own or in any combination, such as "heterocyclalkyl") - a

25 saturated or wholly or partially unsaturated 4-10 membered ring system in which one or more rings contain one or more heteroatoms selected from the group consisting of N, O, or S; such as, but not limited to, pyrrolidine, piperidine, piperazine, morpholine, imidazolidine or pyrazolidine. Such rings as noted herein are included in the R<sub>9</sub> and R<sub>10</sub> optionally cyclized term, as the ring is limited to only 5 to 7 members.

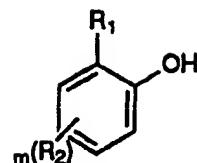
30

The compounds of the present invention may contain one or more asymmetric carbon atoms and may exist in racemic and optically active forms. All of these compounds are included within the scope of the present invention.

35

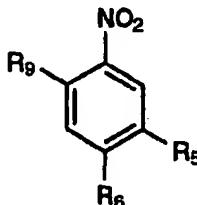
Compounds of Formula (I) and (II) may be prepared as described herein by processes which comprises reacting a suitably protected compound of Formula (2), which are generally commercially available and R<sub>1</sub>, R<sub>2</sub>, m are as defined in Formula (I):

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(2)

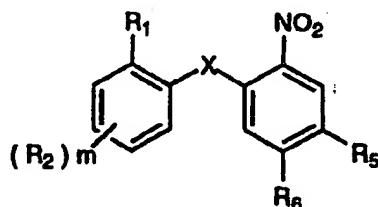
with a compound of Formula (3) wherein R9 is F, Cl, Br or I, and R5 and R6 are as in Formula (I):



5

(3)

in a suitable solvent such as dimethylformamide and in the presence of a suitable base such as potassium carbonate with or without added copper at a temperature of 25-175°C to provide a compound of Formula (4). In cases where compounds of Formula (2) are not commercially available, such as in some cases when R1 is  $\text{S(O)3H}$ , compounds of formula (2) may be prepared by sulfonating an appropriately substituted phenol with a suitable sulfonating reagent, such as fuming sulfuric acid.



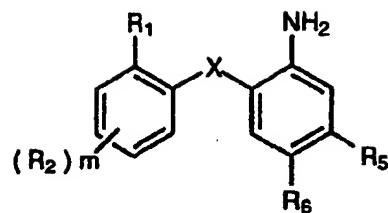
15

(4)

Alternatively, compounds of Formula (4) where R1 is  $\text{-S(O)3H}$  may be prepared by sulfonation of a compound of Formula (4) where R1 is H with a suitable reagent such as fuming sulfuric acid. Further, compounds of Formula (I) wherein R1 is  $\text{-SOCH}_3$  or  $\text{-SO}_2\text{CH}_3$  may be prepared from compounds of Formula (I) wherein R1 is  $\text{-SCH}_3$  by reaction with a suitable oxidizing reagent such as m-chloroperbenzoic acid in a suitable solvent such as methylene chloride.

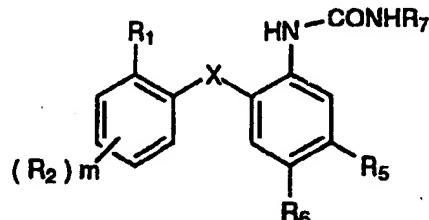
Reduction of compound (4) with a suitable reducing agent such as iron in a solvent such as acetic acid/ethanol/water, or titanium trichloride in acetic acid water, or  $\text{H}_2$  in the presence of a catalyst such as palladium on carbon in a solvent such as ethyl acetate or methanol, provides a compound of Formula (5).

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(5)

Reaction of compound (5) with an isocyanate in a suitable solvent such as pyridine 5 provides a compound of Formula (6) wherein R4 is hydrogen, and R7 is as defined in Formula (I).

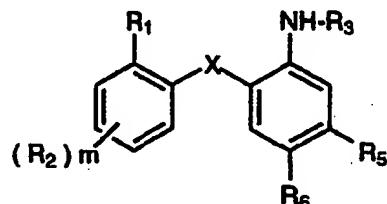


(6)

10

Reaction of a compound of Formula (5) with an alkylating agent or carboxylic acid halide in a suitable solvent such as methylene chloride or pyridine, in the presence of a base, such as triethylamine or pyridine, provides a compound of Formula (8) wherein R3 is C(O)R7 and R7 is an optionally substituted alkyl, aryl or arylalkyl.

15



(8)

Alternatively, compounds of Formula (8), wherein R3 is C(O)R7 may be prepared 20 by reacting a compound of Formula (5) with an appropriate aldehyde under dehydrating conditions to form an imine which may be reduced with a suitable reducing agent such as sodium borohydride.

Deprotection (if required) of a compound of Formulas (6-8) and/or conversion to 25 suitable salt forms provides a final compound of Formula (I).

Without further elaboration, it is believed that one skilled in the art can, using procedures analogous to those described herein, utilize the present invention to its fullest extent. The invention will now be described by reference to the following examples which are merely illustrative and are not to be construed as a limitation of the scope of the present invention.

### SYNTHETIC CHEMISTRY

Temperatures are recorded in degrees centigrade unless otherwise noted.

10

#### Example 1

##### Preparation of 2-[2-(3-Phenylureido)-4-trifluoromethylphenoxy]-5-(1,1-dimethylpropyl)benzenesulfonic acid, sodium salt

15 a) 2-(2-Nitro-4-trifluoromethylphenoxy)-4-(1,1-dimethylpropyl)benzene

A mixture of 4-(1,1-dimethylpropyl)phenol (820 mg, 0.005 mol), 4-bromo-3-nitrobenzotrifluoride (1.35 g, 0.005 mol) and potassium carbonate (1.38 g, 0.01 mol), in dimethylformamide was stirred in an oil bath, at 150°C for sixteen hours. The solvent was evaporated and the residue partitioned between ethyl acetate and water. The phases were separated and the aqueous phase was extracted with ethyl acetate (2x). The combined organic layers were dried over MgSO<sub>4</sub> and evaporated. The residue was flash chromatographed (silica gel, ethyl acetate/hexane) to give the title compound. <sup>1</sup>H NMR (250 MHz, CDCl<sub>3</sub>) δ 8.22 (d, 1H), 7.68 (dd, 1H), 7.48 (dd, 2H), 7.03 (d, 3H), 1.62 (q, 2H), 1.3(s, 6H), 0.70 (t, 3H).

25

b) 2-(2-Nitro-4-trifluoromethylphenoxy)-5-(1,1-dimethylpropyl)benzenesulfonic acid, ammonium salt

Sulfuric acid (15% SO<sub>3</sub>) (10 ml) was added slowly to 2-(2-nitro-4-trifluoromethylphenoxy)-4-(1,1-dimethylpropyl)benzene (7.67 g, 0.02 mol) over approximately an hour, at 0°C. After the addition was complete the mixture was allowed to stir for an hour, at 0°C. The reaction mixture was then quenched in ice water and the aqueous mixture was extracted (3x) with ethyl acetate. The organic phase was dried over MgSO<sub>4</sub>, filtered and evaporated. The residue was flash chromatographed (silica gel, methylene chloride/isopropanol/ammonium hydroxide) to give the title compound - a yellow solid. <sup>1</sup>H NMR (250 MHz, CDCl<sub>3</sub>) δ 7.95 (d, 1H), 7.90 (d, 1H), 7.65 (dd, 1H), 7.39 (dd, 1H), 7.0 (d, 1H), 6.82 (d, 1H), 1.65 (q, 2H), 1.25 (m, 6H), 0.7 (t, 3H).

## c) 2-(2-Amino-4-trifluoromethylphenoxy)-5-(1,1-dimethylpropyl)benzenesulfonic acid

A mixture of 2-(2-nitro-4-trifluoromethylphenoxy)-4-(1,1-dimethylpropyl)-benzenesulfonic acid, ammonium salt (6.82 g 0.015 mol) and 10% palladium on carbon (2.22 g) in ethyl acetate (400 ml) was hydrogenated in a parr bottle at 55 psi, for two hours.

5 The mixture was diluted with chloroform under argon, filtered through Celite® and the filtrate evaporated to give the title compound as a white solid.  $^1\text{H}$  NMR (250 MHz, CD<sub>3</sub>OD)  $\delta$  7.95 (d, 1H), 7.34 (dd, 1H), 7.09 (m, 1H), 6.85 (m, 1H), 6.75 (d, 2H), 1.65 (q, 2H), 1.25 (m, 6H), 0.65 (t, 3H).

10 d) 2-[2-(3-Phenylureido)-4-trifluoromethylphenoxy]-5-(1,1-dimethylpropyl)-benzenesulfonic acid, sodium

2-(2-Amino-4-trifluoromethylphenoxy)-4-(1,1-dimethylpropyl)benzenesulfonic acid (340 mg, 0.0008 mol) and phenyl isocyanate (110  $\mu$ l, 0.0009 mol) were mixed in pyridine (5 ml) at room temperature for sixteen hours. The solvent was evaporated and the residue 15 was flash chromatographed (silica gel, methylene chloride/isopropanol/ammonium hydroxide). The residue was dissolved in water/methanol and sodium bicarbonate was added. The liquid was evaporated and this was repeated one more time. The residue was flash chromatographed (C<sub>18</sub> reverse phase, MeOH/H<sub>2</sub>O) to give the title compound. MS (FAB) m/e 545 [M+H]<sup>+</sup>, m/e 1045 [2M+H]<sup>+</sup>

20

Example 2Preparation of 2-[2-(3-Phenylureido)phenoxy]-5-(1,1-dimethylpropyl)benzenesulfonic acid, sodium salt

25

## a) 2-Hydroxy-5-(1,1-dimethylpropyl)benzenesulfonic acid, ammonium salt

Fuming sulfuric acid (10 mL) and methylene chloride (10 mL) were combined and cooled with stirring to -20°C; and 4-(1,1-dimethylpropyl)phenol (10 g, 6 mmol) was added. After stirring for 15 min, the reaction mixture was quenched on ice and the crude product 30 was purified by flash chromatography (silica gel, methylene chloride/ethanol/ammonium hydroxide) to give the title compound.  $^1\text{H}$  NMR (250 MHz, CD<sub>3</sub>OD/CDCl<sub>3</sub>)  $\delta$  7.65 (d, 1H), 7.26 (dd, 1H), 6.82 (d, 1H), 1.65 (q, 2H), 1.28 (s, 6H), 0.67 (t, 3H).

## b) 2-(2-Nitrophenoxy)-4-(1,1-dimethylpropyl)benzenesulfonic acid, ammonium salt

35 A mixture of 2-hydroxy-5-(1,1-dimethylpropyl)benzenesulfonic acid, ammonium salt (1.1 g, 5.76 mmol), 1-fluoro-2-nitrobenzene (1.2 mL, 8 mmol), and potassium carbonate (1.52 g, 11 mmol) in dimethylformamide (10 mL) was stirred under argon and

heated in an oil bath to 150C° for 90 min. The reaction mixture was diluted with ethyl acetate, filtered, and the residue washed with ethyl acetate. The combined filtrates were evaporated and the crude product was purified by flash chromatography (silica gel, CH<sub>2</sub>Cl<sub>2</sub>/isopropanol/NH<sub>4</sub>OH) to give the title compound. <sup>1</sup>H NMR (250 MHz,

5 CD<sub>3</sub>OD/CDCl<sub>3</sub>) 8.00 (d, 1H), 7.93 (dd, 1H), 7.42-7.55 (m, 2H), 7.20-7.28 (m, 1H), 7.04 (dd, 1H), 6.92 (d, 1H), 1.70 (q, 2H), 1.32 (s, 6H), 0.72 (t, 3H).

c) 2-(2-Aminophenoxy)-4-(1,1-dimethylpropyl)benzenesulfonic acid

A mixture of 2-(2-nitrophenoxy)-4-(1,1-dimethylpropyl)benzenesulfonic acid, 10 ammonium salt (26 g, 0.076 mol) and 10% palladium on carbon (1 g) in ethyl acetate (400 mL) was hydrogenated in a parr bottle at 55 psi for 2 h. The reaction mixture was flushed with argon, filtered through Celite® and evaporated. The crude product was purified by flash chromatography (silica gel, ethyl acetate/hexane) to give the title compound as a white solid.

15 <sup>1</sup>H NMR (250 MHz, CD<sub>3</sub>OD/CDCl<sub>3</sub>) δ 7.93 (d, 1H), 7.27 (dd, 1H), 6.66-7.06 (m, 5H), 1.65 (q, 2H), 1.27 (s, 6H), 0.68 (t, 3H).

d) 2-[2-(3-Phenylureido)phenoxy]-5-(1,1-dimethylpropyl)benzenesulfonic acid, ammonium salt

20 The 2-(2-aminophenoxy)-4-(1,1-dimethylpropyl)benzenesulfonic acid (0.5 g, 1.5 mmol) was dissolved in pyridine (40 mL) and was stirred under argon at room temperature. 3,5-Bis(trifluoromethyl)benzenesulfonyl chloride (0.6 g, 2 mmol) was added and the mixture stirred for 16 h. The solvent was evaporated, and the residue was purified by flash chromatography (silica gel, methylene chloride/ethanol/ammonium hydroxide) to give the 25 title compound. <sup>1</sup>H NMR (250 MHz, CD<sub>3</sub>OD/CDCl<sub>3</sub>) δ 8.18 (dd, 1H), 8.04 (s, 2H), 7.98 (d, 1H), 7.46 (s, 1H), 7.06-7.34 (m, 4H), 6.83 (d, 1H), 1.63 (q, 2H), 1.27 (s, 6H), 0.61 (t, 3H).

e) 2-[2-(3-Phenylureido)phenoxy]-5-(1,1-dimethylpropyl)benzenesulfonic acid, sodium salt

30 A solution of 2-[2-(3-phenylureido)phenoxy]-5-(1,1-dimethylpropyl)benzenesulfonic acid, ammonium salt (0.39 g, 0.62 mmol) in 1:1 methanol/water (10 mL) was stirred under argon. Sodium carbonate (84 mg, 1 mmol) was added and the mixture stirred for 15 min. The solvents were evaporated and the residue was flash chromatographed (C<sub>18</sub> reverse phase, MeOH/H<sub>2</sub>O) to give the title compound after 35 lyophilization. MS (FAB) m/e 477.2 [M+H]<sup>+</sup>

Example 3Preparation of 2-[2-[3-(4-Phenoxyphenyl)ureido]-4-trifluoromethylphenoxy]-5-(1,1-dimethylpropyl)benzenesulfonic acid, sodium salt

5 a) 2-[2-[3-(4-Phenoxyphenyl)ureido]-4-trifluoromethylphenoxy]-5-(1,1-dimethylpropyl)benzenesulfonic acid, sodium salt  
2-(2-Amino-4-trifluoromethylphenoxy)-5-(1,1-dimethylpropyl)benzenesulfonic acid (22 mg, 0.0005 mol) was dissolved in pyridine (5 ml) and stirred under argon. 4-Phenoxyphenylisocyanate (103  $\mu$ l, 0.0006 mol) was added and the reaction was stirred for  
10 sixteen hours. The solvent was evaporated and the residue flash chromatographed (silica gel, methylene chloride/isopropanol/ammonium hydroxide). The fractions containing product were evaporated and the residue mixed with water/methanol and sodium bicarbonate was added. The liquid was evaporated, and this was repeated one time. The residue was flash chromatographed (C 18 reverse phase, MeOH/H<sub>2</sub>O) to give the title compound. MS (FAB) m/e 637 [M+H]<sup>+</sup>, m/e 659 [M+Na]<sup>+</sup>.

Example 4Preparation of 2-[2-(Benzamido)-4-trifluoromethylphenoxy]-5-(1,1-dimethylpropyl)benzenesulfonic acid, sodium salt

20 a) 2-[2-Benzamido)-4-trifluoromethylphenoxy]-5-(1,1-dimethylpropyl)benzenesulfonic acid, sodium salt

Following the procedure of Example 3 except substituting benzoyl chloride for 4-phenoxyphenylisocyanate, the title compound was obtained. MS (FAB) m/e 530 [M+H]<sup>+</sup>, m/e 552 [M+Na]<sup>+</sup>.

Example 530 Preparation of 2-[2-[3,5-Bis (trifluoromethyl)benzamido]-4-trifluoromethylphenoxy]-5-(1,1-dimethylpropyl)benzenesulfonic acid, sodium salt

a) 2-[2-[3,5-Bis(trifluoromethyl)benzoamido]-4-trifluoromethylphenoxy]-5-(1,1-dimethylpropyl)benzenesulfonic acid, sodium salt

35 Following the procedure of Example 3 (a) except substituting 3,5-bis(trifluoromethyl)benzoyl chloride for 4-phenoxyphenyl isocyanate, the title compound was obtained. MS (FAB) m/e 688 [M+Na]<sup>+</sup>, m/e 1353 [2M+Na]<sup>+</sup>.

Example 6Preparation of 2-[2-[3-[3,5-Bis (trifluoromethyl)phenylureido]-4-trifluoromethylphenoxy]-5-(1,1,3,3-tetramethylbutyl)benzenesulfonic acid, sodium salt

5

a) 2-(2-Nitro-4-trifluoromethylphenoxy)-4-(1,1,3,3-tetramethylbutyl)benzene

A mixture of 4-(*tert*-octyl)phenol (500 mg, 0.0024 mol), 4-bromo-3-nitrobenzotrifluoride (648 mg, 0.0024 mol) and potassium carbonate (663 mg, 0.0048 mol) in dimethylformamide (25 ml) was stirred in an oil bath, at 150°C for sixteen hours. The cooled mixture was filtered and the filtrate evaporated. The residue was flash chromatographed (silica gel, ethyl acetate/hexane) to give the title compound.  $^1\text{H}$  NMR (250 MHz, CD<sub>3</sub>OD)  $\delta$  8.25 (d, 1H), 7.8 (dd, 1H), 7.5 (tt, 2H), 7.05 (m, 3H), 1.85 (s, 2H), 1.4 (s, 6H), 0.75 (s, 9H).

10

15 b) 2-(2-Nitro-4-trifluoromethylphenoxy)-5-(1,1,3,3-tetramethylbutyl)benzenesulfonic acid, ammonium salt

2-(2-Nitro-4-trifluoromethylphenoxy)-4-(1,1,3,3-tetramethylbutyl)benzene (590 mg, 0.0015 mol) was dissolved in methylene chloride and slowly dripped into a 1:1 mixture of sulfuric acid/methylene chloride at 0°C. After the addition was complete the mixture was stirred for ten minutes at 0°C, noting a color change - yellow to purple. The reaction mixture was quenched in ice and the aqueous mixture extracted with ethyl acetate (3x). The organic phase was dried over MgSO<sub>4</sub>, filtered, and evaporated. The crude product was flash chromatographed (silica gel, methylene chloride/isopropanol/ammonium hydroxide) to give the title compound.  $^1\text{H}$  NMR (250 MHz, CD<sub>3</sub>OD)  $\delta$  8.2 (d, 1H), 8.07 (d, 1H), 7.72 (dd, 1H), 7.52 (dd, 1H), 7.0 (m, 2H), 1.82 (s, 2H), 1.4 (s, 6H), 0.8 (s, 9H).

20

25 c) 2-(2-Amino-4-trifluoromethylphenoxy)-5-(1,1,3,3-tetramethylbutyl)benzenesulfonic acid, ammonium salt

2-(2-Nitro-4-trifluoromethylphenoxy)-5-(1,1,3,3-tetramethylbutyl)benzenesulfonic acid, ammonium salt (650 mg, 0.0013 mol) was dissolved in ethyl acetate (30 ml) and 10% palladium on carbon (125 mg) was added. The mixture was hydrogenated at 55 psi, in a parr bottle, for three hours. The reaction mixture was diluted with chloroform under argon, filtered through Celite® and the filtrate evaporated. The residue was flash chromatographed (silica gel, methylene chloride/isopropanol/ammonium hydroxide) to give the title compound.  $^1\text{H}$  NMR (250 MHz, CD<sub>3</sub>OD)  $\delta$  8.0 (d, 1H), 7.35 (dd, 1H), 7.05 (m, 2H), 6.9 (dd, 1H), 6.75 (d, 1H), 1.75 (s, 2H), 1.40 (s, 6H), 0.75 (s, 9H).

-19-

d) 2-[2-[3-[3,5-Bis(trifluoromethyl)phenoxy]ureido]-4-trifluoromethylphenoxy]-5-(1,1,3,3-tetramethylbutyl)benzenesulfonic acid, sodium salt

A mixture of 2-(2-amino-4-trifluoromethylphenoxy)-5-(1,1,3,3-tetramethylbutyl)benzenesulfonic acid, ammonium salt (440 mg, 0.001 mol), 3,5-

5 bis(trifluoromethyl)phenyl isocyanate (364  $\mu$ l, 0.0022 mol) and pyridine (12 ml) was stirred at room temperature for sixteen hours. The solvent was evaporated and the residue flash chromatographed (silica gel, methylene chloride/isopropanol/ammonium hydroxide). The residue was dissolved in water/methanol and sodium bicarbonate was added and the liquid evaporated. This was repeated to give title compound. MS (ES) m/e 701 [M+H]<sup>+</sup>, m/e 723

10 [M+Na]<sup>+</sup>.

#### Example 7

Preparation of 2-[2-[3-[3,5-Bis (trifluoromethyl)phenylureido]-4-methylphenoxy]-5-(1,1-dimethylpropyl)benzenesulfonic acid, sodium salt

a) 2-[2-[3,5-Bis(trifluoromethyl)phenylureido]-4-methylphenoxy]-5-(1,1-dimethylpropyl)benzenesulfonic acid, sodium salt

Following the procedure of Example 3 except substituting 3,5-

20 bis(trifluoromethyl)phenyl isocyanate for 4-phenoxyphenyl isocyanate, the title compound was obtained. MS (ES) m/e [M+2H-Na]<sup>+</sup>, m/e 627 [M+H]<sup>+</sup>

#### Example 8

25 Preparation of 2-[2-[3-[3,5-Bis (trifluoromethyl)phenylureido]-4-trifluoromethylphenoxy]benzenesulfonic acid, sodium salt

a) 2-(2-Nitro-4-trifluoromethylphenoxy)benzenesulfonic acid, ammonium salt

A mixture of 2-hydroxybenzenesulfonic acid, ammonium salt (500 mg, 0.0026

30 mol), 4-fluoro-3-nitrobenzotrifluoride (746  $\mu$ l, 0.0053 mol) and potassium carbonate (719 mg, 0.005 mol) in dimethylformamide (20 ml) was stirred at 150°C, in an oil bath, for sixteen hours. The solvent was evaporated and the residue flash chromatographed (silica gel, methylene chloride/isopropanol/ammonium hydroxide) to give the title compound. <sup>1</sup>H NMR (250 MHz, CDCl<sub>3</sub>)  $\delta$  8.25 (s, 1H), 8.05 (d, 1H), 7.7 (dd, 1H), 7.5 (m, 1H), 7.35 (t, 1H), 7.08 (m, 2H).

b) 2-(2-Amino-4-trifluoromethylphenoxy)benzenesulfonic acid, ammonium salt

2-(2-Nitro-4-trifluoromethylphenoxy)benzenesulfonic acid, ammonium salt (670 mg) was dissolved in ethyl acetate (20 ml) and 10% palladium on carbon (335 mg) was added under argon. The mixture was hydrogenated, in a part bottle, at 55 psi for three hours. The reaction mixture was diluted with chloroform under argon, filtered through 5 Celite® and the filtrate evaporated. The residue was flash chromatographed (silica gel, methylene chloride/isopropanol/ammonium hydroxide) to give the title compound.  $^1\text{H}$  NMR (250 MHz,  $\text{CDCl}_3$ )  $\delta$  8.0 (dd, 1H), 7.35 (t, 2H), 7.1 (m, 2H), 6.95 (dd, 1H), 6.82 (d, 1H).

10 c) 2-[2-[3,5-Bis(trifluoromethyl)phenyl]ureido]-4-(trifluoromethyl)phenoxy]-benzenesulfonic acid, sodium salt

A mixture of 2-(2-amino-4-trifluoromethylphenoxy)benzenesulfonic acid, ammonium salt (400 mg, 0.0011 mol) and 3,5-di(trifluoromethyl)phenyl isocyanate in pyridine (10 mL) was stirred at room temperature for sixteen hours. The solvent was 15 evaporated and the residue flash chromatographed (silica gel, methylene chloride/isopropanol/ammonium hydroxide). The fractions containing product were evaporated and the residue dissolved in water/methanol and sodium bicarbonate added. The liquid was evaporated and this was repeated one time to give the title compound.  $^1\text{H}$  NMR (500 MHz,  $\text{CD}_3\text{OD}$ )  $\delta$  8.85 (d, 1H), 8.1 (s, 2H), 7.95 (dd, 1H), 7.55 (s, 1H), 7.40 (dd, 20 1H), 7.35 (tt, 1H), 7.28 (d, 1H), 7.15 (dt, 1H), 6.95 (dd, 1H).

Example 9

Preparation of 2-[2-[3,5-Bis(trifluoromethyl)phenyl]methylacetamido]-4-(trifluoromethyl)phenoxy]-5-(1,1-dimethylpropyl)benzenesulfonic acid, sodium salt

25

a) 3,5-bis(trifluoromethyl)phenyl acetyl chloride

3,5-bis(trifluoromethyl)phenylacetic acid (1.09 g, 4 mmol) was dissolved in methylene chloride (10 mL) at -10°C and stirred under argon. Oxalyl chloride (0.436 mL, 5 mmol) and dimethylformamide (0.01 mL) were added and the mixture stirred until gas 30 evolution ceased. The solvents were evaporated and the crude product was used in the next step without further purification.

b) 2-[2-[3,5-Bis(trifluoromethyl)phenylmethylacetamido]-4-(trifluoromethyl)phenoxy]-5-(1,1-dimethylpropyl)benzenesulfonic acid, ammonium salt

35

2-(2-amino-4-trifluoromethylphenoxy)-5-(1,1-dimethylpropyl)benzenesulfonic acid, ammonium salt (0.3 g, 0.74 mmol) was dissolved in methylene chloride (4 mL) and stirred under argon at -40°C. Pyridine (0.061 mL, 0.75 mmol) was added followed by the

dropwise addition of 3,5-bis(trifluoromethyl)phenyl acetyl chloride (232 mg, 0.8 mmol) in methylene chloride (1 mL). The solvent was evaporated and the crude product was purified by flash chromatography (silica gel, methylene chloride/isopropanol/ammonium hydroxide) to give the title product.  $^1\text{H}$  NMR (250 MHz,  $\text{CDCl}_3$ )  $\delta$  8.62-8.64 (d, 1H), 8.00-8.02 (d, 1H), 7.96 (s, 2H), 7.81 (s, 1H), 7.31-7.46 (m, 3H), 6.88-6.92 (d, 1H).

5 c) 2-[2-[3,5-Bis(trifluoromethyl)phenylmethylacetamido]-4-(trifluoromethyl)phenoxy]-5-(1,1-dimethylpropyl)benzenesulfonic acid, sodium salt

A solution of 2-[2-[3,5-bis(trifluoromethyl)phenylmethylacetamido]-4-(trifluoromethyl)phenoxy]-5-(1,1-dimethylpropyl)benzenesulfonic acid, ammonium salt (0.28 g, 0.41 mmol) in 1:1 methanol/water (10 mL) was stirred under argon. Sodium carbonate (55 mg, 0.65 mmol) was added and the mixture stirred for 15 min. The solvents were evaporated and the residue was flash chromatographed ( $\text{C}_{18}$  reverse phase,  $\text{MeOH}/\text{H}_2\text{O}$ ) to give the title compound after lyophilization. MS (FAB) m/e 680.4  $[\text{M}+\text{H}]^+$ .

10 15 The following compounds may also be made by one of skill in the art using methods analogous to the Examples indicated above.

Example 10: 2-[2-(Phenylmethylacetamido)phenoxy]-5-(1,1-dimethylpropyl)-  
20 benzenesulfonic acid

Example 11: 2-[2-[3,5-bis(trifluoromethyl)phenyl]thioureido]-4-(trifluoromethyl)phenoxy]-5-(1,1-dimethylpropyl)benzenesulfonic acid

Example 12: 2-[2-[3-(4-Methoxyphenyl)ureido]-4-trifluoromethylphenoxy]-5-(1,1-dimethylpropyl)-benzenesulfonic acid

25 Example 13: 2-[2-[3,5-Bis(trifluoromethyl)phenyl]ureido]phenoxy]-5-(1,1-dimethylpropyl)-benzenesulfonic acid, and its sodium salt.

Example 14: 2-[2-(Nonanamido)phenoxy]benzoic acid; m.p. 81-82 °C

Example 15: 2-[2-[3,5-Bis-(trifluoromethyl)]benzamido-4-trifluoromethylphenoxy]-benzoic acid; m.p. 178 °C

### 30 METHODS OF TREATMENT

The compounds of Formula (I)/(II), or pharmaceutically acceptable salts thereof can be used in the manufacture of a medicament for the prophylactic or therapeutic treatment of an inflammatory disease state in a mammal, preferably a human.

35 Inhibition of PLA<sub>2</sub> and/or CoA-IT and the simultaneous reduction of PAF, free arachidonic acid and eicosanoid release from inflammatory cells according to this invention is of therapeutic benefit in a broad range of diseases or disorders. The invention herein is

therefore useful to treat such disease states both in humans and in other mammals.

Inhibition of CoA-IT and PLA<sub>2</sub> by the compounds of Formula (I)/(II) is an effective means for simultaneously reducing PAF, free arachidonic acid and eicosanoids produced in inflammatory cells. The therapeutic utility of blocking lipid mediator generation has been 5 recognized for many years. For example, inhibitors of cyclooxygenase, such as aspirin, indomethacin, acetaminophen and ibuprofen, have demonstrated broad therapeutic utilities. CoA-IT inhibitors inhibit cyclooxygenase products. Another class of inhibitors which are used in a broad range of inflammatory disorders are the corticosteroids. Corticosteroids act 10 in a variety of ways, e.g. to induce inflammatory cells to produce proteins which inhibit free arachidonic acid release or to down regulate PLA<sub>2</sub> mRNA formation. CoA-IT inhibitors block the release of free arachidonic acid. Inhibitors of 5-lipoxygenase block the production of leukotrienes and leukotriene antagonists prevent the bioactions of leukotrienes. Recent studies indicate that both will have broad therapeutic utilities, and CoA-IT inhibitors block 15 the production of leukotrienes. Inhibitors of phospholipase A<sub>2</sub> block the release of free arachidonic acid and the formation of lyso PAF (the immediate precursor of PAF). PLA<sub>2</sub> inhibitors are recognized to have broad therapeutic utilities. It does not, however, follow that the disease states noted above are in fact caused by altered CoA-IT activity or PLA<sub>2</sub>. Thus, the disease state itself may not be directly mediated by CoA-IT or PLA<sub>2</sub> activity. It 20 only follows that CoA-IT or PLA<sub>2</sub> activity is required for the continued expression of symptoms of the disease state and that CoA-IT or PLA<sub>2</sub> inhibitors will be beneficial against the symptoms of these disease states.

Recognition that CoA-IT reduces PAF production has a number of therapeutic implications. PAF itself has been implicated as being involved in a number of medical conditions. Thus in circulatory shock, which is characterised by systemic hypotension, 25 pulmonary hypertension and increased lung vascular permeability, the symptoms can be mimicked by infusion of PAF. This coupled with evidence showing that circulating PAF levels are increased by endotoxin infusion indicate that PAF is a prime mediator in certain forms of shock.

Intravenous infusion of PAF at doses of 20-200 pmol kg<sup>-1</sup> min<sup>-1</sup> into rats 30 has been reported to result in the formation of extensive haemorrhagic erosions in the gastric mucosa. Thus PAF is the most potent gastric ulcerogen yet described whose endogenous release may underlie or contribute to certain forms of gastric ulceration. Psoriasis is an inflammatory and proliferative disease characterised by skin lesions. PAF is pro-inflamatory and has been isolated from lesioned scale of psoriatic patients indicating PAF 35 has a role in the disease of psoriasis. And finally, increasing evidence supports a potential patho-physiological role for PAF in cardiovascular disease. Thus recent studies in angina patients show PAF is released during atrial pacing. Intracoronary injection of PAF in pigs

induces a prolonged decrease in coronary flow and, in guinea pig hearts, it induces regional shunting and ischaemia. In addition PAF has been shown to initiate thrombus formation in a mesenteric artery preparation, both when administered exogenously and when released endogenously. More recently PAF has been shown to play a role in brain ischaemia

5 induced in animal models of stroke.

Thus the compounds of the invention, by virtue of their ability to antagonise either CoA-IT and/or PLA<sub>2</sub>, thus block the production of PAF, free arachidonic acid and its metabolites, are likely to be of value in the treatment of any of the above conditions.

Disease states which could benefit from the inhibition of lipid mediator production  
10 include, but are not limited to, adult respiratory distress syndrome, asthma, arthritis, reperfusion injury, endotoxic shock, inflammatory bowel disease, allergic rhinitis and various inflammatory skin disorders. Each of these disorders is mediated in some part by lipid mediators of inflammation. Compounds which inhibit CoA-IT, by virtue of their ability to block the generation of lipid mediators of inflammation, are of value in the  
15 treatment of any of these conditions. Similarly compounds which inhibit PLA<sub>2</sub>, by virtue of their ability to block the generation of lipid mediators of inflammation stemming from release of this enzyme are of value in the treatment of these conditions. In particular, an inhibitor of PLA<sub>2</sub>, for instance would offer an advantage over the classical NSAIDs which affect only prostanoid production (and not PAF biosynthesis) thereby inhibiting both the  
20 acute and cell-mediated "chronic" inflammatory processes. Likewise, selective inhibition of COX-2, by compounds herein, is useful for the treatment of diseases mediated thereby, such as arthritis, and IBD, for relief of pain, and inflammation, without the gastric and renal side effects associated with COX-1 inhibition.

Treatment of disease states caused by these lipid inflammatory mediators i.e.,  
25 arachidonate, eicosanoids and PAF, include certain cardiovascular disorders such as but not limited to, myocardial infarction, stroke, circulatory shock, or hypotension, ischemia, reperfusion injury, inflammatory diseases such as, but not limited to, arthritis, inflammatory bowel disease, Crohn's disease, or ulcerative colitis, respiratory disease such as but not limited to, asthma, or adult respiratory distress syndrome, anaphylaxis, shock such as but not  
30 limited to endotoxic shock, topical diseases, such as but not limited to actinic keratosis, psoriasis, or contact dermatitis, or pyresis.

In order to use a compound of formula (I) or (II) a pharmaceutically acceptable salt thereof in therapy, it will normally be formulated into a pharmaceutical composition in  
35 accordance with standard pharmaceutical practice. This invention, therefore, also relates to a pharmaceutical composition comprising an effective, non-toxic amount of a compound of formula (I) and a pharmaceutically acceptable carrier or diluent.

Compounds of formula (I)/(II), pharmaceutically acceptable salts thereof and pharmaceutical compositions incorporating such may conveniently be administered by any of the routes conventionally used for drug administration, for instance, orally, topically, parenterally or by inhalation. The compounds of formula (I)/(II) may be administered in 5 conventional dosage forms prepared by combining a compound of formula (I)/(II), with standard pharmaceutical carriers according to conventional procedures. Such pharmaceutically acceptable carriers or diluents and methods of making are well known to those of skill in the art, and reference can be found in such texts as Remington's Pharmaceutical Sciences, 18th Ed., Alfonso R. Genarao, Ed., 1990, Mack Publishing Co. 10 and the Handbook of Pharmaceutical Excipients, APhA Publications, 1986.

The compounds of formula (I)/(II) may also be administered in conventional dosages in combination with known second therapeutically active compounds, such as steroids or NSAID's for instance. These procedures may involve mixing, granulating and compressing or dissolving the ingredients as appropriate to the desired preparation. It will 15 be appreciated that the form and character of the pharmaceutically acceptable carrier or diluent is dictated by the amount of active ingredient with which it is to be combined, the route of administration and other well-known variables. The carrier(s) must be "acceptable" in the sense of being compatible with the other ingredients of the formulation and not deleterious to the recipient thereof.

20 The pharmaceutical carrier employed may be, for example, either a solid or liquid. Exemplary of solid carriers are lactose, terra alba, sucrose, talc, gelatin, agar, pectin, acacia, magnesium stearate, stearic acid and the like. Exemplary of liquid carriers are syrup, peanut oil, olive oil, water and the like. Similarly, the carrier or diluent may include time delay material well known to the art, such as glyceryl mono-stearate or glyceryl distearate alone or 25 with a wax.

A wide variety of pharmaceutical forms can be employed. Thus, if a solid carrier is used, the preparation can be tableted, placed in a hard gelatin capsule in powder or pellet form or in the form of a troche or lozenge. The amount of solid carrier will vary widely but preferably will be from about 25mg. to about 1g. When a liquid carrier is used, the 30 preparation will be in the form of a syrup, emulsion, soft gelatin capsule, sterile injectable liquid such as an ampule or nonaqueous liquid suspension.

Compounds of formula (I)/(II) may be administered topically, that is by non-systemic administration. This includes the application of a compound of Formula (I)/(II), externally to the epidermis or the buccal cavity and the instillation of such a compound into 35 the ear, eye and nose, such that the compound does not significantly enter the blood stream. In contrast, systemic administration refers to oral, intravenous, intraperitoneal and intramuscular administration.

Formulations suitable for topical administration include liquid or semi-liquid preparations suitable for penetration through the skin to the site of inflammation such as liniments, lotions, creams, ointments or pastes, and drops suitable for administration to the eye, ear or nose. The active ingredient may comprise, for topical administration, from 5 0.001% to 10% w/w, for instance from 1% to 2% by weight of the formulation. It may however comprise as much as 10% w/w but preferably will comprise less than 5% w/w, more preferably from 0.1% to 1% w/w of the formulation.

Lotions according to the present invention include those suitable for application to the skin or eye. An eye lotion may comprise a sterile aqueous solution optionally containing 10 a bactericide and may be prepared by methods similar to those for the preparation of drops. Lotions or liniments for application to the skin may also include an agent to hasten drying and to cool the skin, such as an alcohol or acetone, and/or a moisturizer such as glycerol or an oil such as castor oil or arachis oil.

Creams, ointments or pastes according to the present invention are semi-solid 15 formulations of the active ingredient for external application. They may be made by mixing the active ingredient in finely-divided or powdered form, alone or in solution or suspension in an aqueous or non-aqueous fluid, with the aid of suitable machinery, with a greasy or non-greasy base. The base may comprise hydrocarbons such as hard, soft or liquid paraffin, glycerol, beeswax, a metallic soap; a mucilage; an oil of natural origin such as 20 almond, corn, arachis, castor or olive oil; wool fat or its derivatives or a fatty acid such as steric or oleic acid together with an alcohol such as propylene glycol or a macrogel. The formulation may incorporate any suitable surface active agent such as an anionic, cationic or non-ionic surfactant such as a sorbitan ester or a polyoxyethylene derivative thereof. Suspending agents such as natural gums, cellulose derivatives or inorganic materials such as 25 silicaceous silicas, and other ingredients such as lanolin, may also be included.

Drops according to the present invention may comprise sterile aqueous or oily 30 solutions or suspensions and may be prepared by dissolving the active ingredient in a suitable aqueous solution of a bactericidal and/or fungicidal agent and/or any other suitable preservative, and preferably including a surface active agent. The resulting solution may then be clarified by filtration, transferred to a suitable container which is then sealed and sterilized by autoclaving or maintaining at 98-100 °C. for half an hour. Alternatively, the 35 solution may be sterilized by filtration and transferred to the container by an aseptic technique. Examples of bactericidal and fungicidal agents suitable for inclusion in the drops are phenylmercuric nitrate or acetate (0.002%), benzalkonium chloride (0.01%) and chlorhexidine acetate (0.01%). Suitable solvents for the preparation of an oily solution include glycerol, diluted alcohol and propylene glycol.

Each dosage unit for oral administration contains preferably from 1 to 250 mg (and

for parenteral administration contains preferably from 0.1 to 25 mg) of a compound of the Formula (I) or a pharmaceutically acceptable salt thereof calculated as the free base.

The pharmaceutically acceptable compounds of the invention will normally be administered to a subject in a daily dosage regimen. For an adult patient this may be, for 5 example, an oral dose of between 1 mg and 500 mg, preferably between 1 mg and 250 mg, or an intravenous, subcutaneous, or intramuscular dose of between 0.1 mg and 100 mg, preferably between 0.1 mg and 25 mg, of the compound of the Formula (I)/(II), or a pharmaceutically acceptable salt thereof calculated as the free base, the compound being administered from 1 to 4 times per day.

10 It will be recognized by one of skill in the art that the optimal quantity the individual dosages of the compounds used herein will be determined by the nature and extent of the condition being treated, the form, route and site of administration, and the particular patient being treated, and that such optimums can be determined by conventional techniques by those skilled in art. The dosage to be administered is obviously dependent upon species, 15 body weight, age, individual condition and method of application. It will also be appreciated by one of skill in the art that the optimal course of treatment, i.e., the number of doses given per treatment, the frequency of such treatment and the eventual duration of use can be ascertained by those skilled in the art using conventional course of treatment determination tests.

20

#### BIOLOGICAL METHODS:

To determine activity of the compounds of Formula (I) and (II) various cellular assays can be used to determine in vitro activity. Additionally, various classical in vivo acute inflammatory models which have some aspect of their etiology to elevated eicosanoid 25 levels can be employed, such as the paw edema model, mouse zymosan peritonitis, reverse Arthus pleurisy or various skin inflammation assays which are described in Lewis et al., Experimental Models of Inflammation, in the Handbook of Inflammation, Vol. 5, Bonta Ed., Elsevier Science Publishers, NY (1985) whose disclosure is herein incorporated by reference. The TPA induced ear edema model (mouse) as well as the carrageenan paw 30 edema model in the rat are described herein as well. These classical models of inflammation will reflect the drug's ability to alter an inflammatory response but cannot address the specificity of drug action. These models have been traditionally designed as non steriod antiinflammatory drug sensitive pharmacological screens and it is important to utilize models which can differentiate PLA<sub>2</sub> and CoA-IT inhibitors from NSAIDS.

35

#### Cell-free and Cellular Assessment of Inhibitors

Described herein are several in vitro assays both for CoA-IT and PLA<sub>2</sub> enzyme activities.

The first employs purified recombinant enzyme or a broken cell assay, assay (a or b, respectively) described below. Alternatively, evaluation of inhibitors can occur in intact cells such as described in the assay, assay (c and d) below. CoA-IT activity can exclusively be measured, and differentiated from PLA<sub>2</sub> inhibition, in intact cells by following the movement of a pulse of [<sup>3</sup>H] arachidonate as it moves into the 1-alkyl and 1-alkenyl phospholipids in inflammatory cells (assay e). It should be noted for the purposes herein that assays c, d, & f can both be used for PLA<sub>2</sub> and CoA-IT inhibition determination.

**10** Inflammatory Responses *in vivo*  
The ability of compounds that inhibit CoA-IT and/or PLA<sub>2</sub> to affect *in vivo* inflammatory responses may be assessed. Inflammatory responses are induced in the mouse ear by the topical application of a pro-inflammatory agent, such as 12-O-tetradecanoylphorbol 13-acetate (assay g). This produces an edematous response, as measured by increases in ear thickness, as well as increased inflammatory cellular infiltrate.

**15** as measured by increases in myeloperoxidase activity (as described in the methods). To further validate the mechanism of action inflammation induced by the direct administration of arachidonic acid can be used. In this case, compounds altering arachidonic acid mobilization or liberation should be without effect.

**20** *In Vitro Assays*

**25** Assay (a): Phospholipase A<sub>2</sub> assay:  
Phospholipase A<sub>2</sub> activity of rh Type II - 14 KDaPLA<sub>2</sub> or PLA<sub>2</sub> semi-purified from human Synovial joint fluid was measured by the acylhydrolysis of high specific activity (NEN)[<sup>3</sup>H]-AA-*E. coli* (0.5 mCi/Snmol PL Pi) as previously described in Marshall et al., J. Rheumatology, 18:1, pp59-65 (1991). High specific activity [<sup>3</sup>H]AA-*E. coli* had up to 95% of the label incorporated into phospholipid which was localized almost exclusively in the sn-2 position, as demonstrated by purified 14kDa PLA<sub>2</sub> or low molecular weight PLA<sub>2</sub> acylhydrolysis and separation of products by thin layer chromatography (TLC) (data not shown). [Predominately used herein was rh Type II 14 kDa PLA<sub>2</sub>, or alternatively bovine pancreatic PLA<sub>2</sub> was also be used]. The reaction mixture (50 or 100 ml total volume) contained 25 mM HEPES, pH 7.4, 150 mM NaCl, 5 mM CaCl<sub>2</sub> and [<sup>3</sup>H]-AA-*E. coli* (low specific activity; 5-8 nmol PL Pi per assay). Assays were incubated for a time predetermined to be on the linear portion of a time versus hydrolysis plot (10 min).

**30** Experiments were conducted with final % hydrolysis values ranging from 2% (400-1000 dpm) to 10% (2000-5000 dpm) acylhydrolysis after blank correction. Reactions were terminated by the addition of 1.0 mL tetrahydrofuran (THF). The whole sample was placed

over aminopropyl solid phase silica columns and eluted with THF:acetic acid (49:1) exclusively separating free fatty acids with greater than 95% recovery. Radiolabel in this eluate was quantitated by liquid scintillation counting. Results were expressed as % of fatty acid hydrolyzed ([sample dpms - non-specific (blank) dpms/total dpms] x 100) or specific activity which was calculated from hydrolysis values found in the linear portion of time versus % hydrolysis plots (pmol free fatty acid hydrolyzed/mg/min). Non-specific activity was always less than 1% of the total counts added.

Protein determination

1 0 All protein concentrations were determined by Bradford protein analysis kits (Biorad, Richmond, CA).

Results:

1 5 The following representative compounds of Formula (I) and (II) below demonstrated positive PLA<sub>2</sub> inhibition in the above noted method. While these compounds generally tested positive at 50  $\mu$ M levels, several were also tested for positive inhibitory activity at up to 500  $\mu$ M levels. Such compounds include all compounds of Examples 1 to 13 herein.

2 0 Assay (b) : CoA-IT Activity

The following is a method to measure CoA-IT activity and the effects of compounds on CoA-IT activity. The assay is based upon mixing cellular material containing CoA-IT activity with a stable lyso phospholipid such as 1-alkyl-2-acyl-GPC and measuring the production of phospholipid product such as 1-alkyl-2-acyl-GPC occurring in the absence of 2 5 added CoA or CoA-fatty acids.

Cell Preparation

Any inflammatory cell that contains high levels of CoA-IT activity can be used, such as neutrophils, macrophages or cell lines such as U937 cells. U937 cells were obtained 3 0 from American Type Culture Collection and grown in RPMI-1640 media (Gibco, Grand Island, New York) supplemented with 10% fetal bovine serum (Hyclone, Logan, UT) at 37°C, 5% CO<sub>2</sub>. Cells were grown without differentiation (basal state) by any agent, such as dimethyl sulfoxide. As used herein, "inflammatory cells" include, but are not limited to neutrophils, macrophages, monocytes, lymphocytes, eosinophils, basophils, and mast 3 5 cells.

Microsomal preparation

Microsomes were prepared using standard techniques. In this case, cells were washed with a buffer of 250 mM sucrose, 10 mM Tris, 1 mM EGTA, 1 mM MgCl<sub>2</sub>, pH 7.4 and ruptured by N<sub>2</sub> cavitation (750 psi, 10 minutes). The ruptured cells were centrifuged 1000 X g, 5 minutes. The resulting supernatant was centrifuged at 20,000 X g, ~20 minutes. Microsomes were prepared from this supernatant by centrifugation at 100,000 x g, 60 minutes. The resulting pellet was washed once with assay buffer (150 mM NaCl, 10 mM Na<sub>2</sub>KPO<sub>4</sub>, 1 mM EGTA, pH 7.4), re-centrifuged and the pellet resuspended in assay buffer (4-20 mg protein/ml) and was stored at -80°C until assayed.

10        CoA-IT activity

CoA-IT activity was measured in 1.5 ml centrifuge tubes in a total volume of 100 ul. Microsomes were diluted in assay buffer to the desired protein concentration (6-20 ug/tube). The reaction was initiated by addition of [<sup>3</sup>H] 1-alkyl-2-lyso-sn-glycero-3-phosphocholine (GPC) (~ 0.1 uCi/tube) and 1  $\mu$ M final cold 1-alkyl-2-lyso-GPC in assay buffer with 0.25 mg/ml fatty acid-poor bovine serumalbumin (BSA) (Calbiochem, La Jolla, CA). [<sup>3</sup>H]1-alkyl-2-lyso-GPC, approximately 50 Ci/mmol, was from NEN-Dupont (Boston, Massachusetts) and cold 1-alkyl-2-lyso-GPC was from Biomol (Plymouth Meeting, Pennsylvania). Microsomes were pretreated with desired agents for the desired time (10 minutes) before the addition of [<sup>3</sup>H]1-alkyl-2-lyso-GPC. The reaction was run for the desired time (10 minutes) at 37°C. The reaction was stopped and the lipids extracted by addition of 100 ul of chloroform:methanol (1:2, v/v) followed by 100 ul of chloroform and 100 ul of 1 M KCl. The samples were vortexed and centrifuged at high speed in a microfuge for 2-3 minutes. An aliquot of the chloroform-extracted materials were separated, usually by TLC in chloroform/methanol/acetic acid/water (50:25:8:4, v/v), visualized by radioscaning (Bioscan) and the product, [<sup>3</sup>H] 1-alkyl-2-acyl-GPC, was scraped and quantified by liquid scintillation spectroscopy. With this TLC system, the synthetic standards of 1-alkyl-2-lyso-GPC and 1-alkyl-2-acyl-GPC were well separated, with R<sub>f</sub> values of approximately 0.25 and 0.65, respectively. Other methods can be used to separate substrate from product, including but not limited to column chromatography, affinity chromatography and post reaction derivitization.

Protein concentration were assessed using the protein assay reagents from Bio-Rad (Richmond, California).

Results

35        A variety of compounds have been tested in this assay to determine its selectivity and inability to detect trivial, non-selective inhibitors. Inhibitors of 5-lipoxygenase (5-LO) and cyclooxygenase (CO), such as indomethacin, naproxen, 6-(4'-Fluorophenyl)-5-(4-pyridyl)-

2,3-dihydroimidzo-[2,1-b]thiazole and 6-(4'-Fluorophenyl)-5-(4-pyridyl)2,3-dihydroimidzo-[2,1-b]thiazole-dioxide had no effect on CoA-IT activity at concentrations up to 100  $\mu$ M. The anti-oxidant BHT also has no effect at concentrations up to 100  $\mu$ M. Compounds which complex with phospholipids and inhibit PLA<sub>2</sub> activity, such as 5 quinacrine and aristolochic acid have no effect on CoA-IT activity at concentrations up to 500  $\mu$ M. Doxepine, a compound reported to inhibit PAF release did not inhibit CoA-IT at concentrations up to 100  $\mu$ M. Sodium diclofenac, reported to decrease leukotriene production by altering arachidonic acid metabolism, had no effect on CoA-IT activity at 10 concentrations up to 500  $\mu$ M. These results show that the assay for CoA-IT activity is sensitive and selective.

Representative compounds of Formula (I) and (II) Representative compounds of Formula (I) which inhibit CoA-IT activity in the microsomal CoA-IT assay (assay (a) above) [generally at 50  $\mu$ M or less] are the compounds:

15 2-[2-(3-Phenylureido)phenoxy]-5-(1,1-dimethylpropyl)benzenesulfonic acid; 2-[2-(3-Phenylureido)-4-trifluoromethylphenoxy]-5-(1,1-dimethylpropyl)benzenesulfonic acid; 2-[2-[3-(4-Phenoxyphenyl)ureido]-4-trifluoromethylphenoxy]-5-(1,1-dimethylpropyl)-benzenesulfonic acid; 20 2-[2-[3-[3,5-Bis(trifluoromethyl)phenyl]ureido]-4-trifluoromethylphenoxy]-5-(1,1,3,3-tetramethylbutyl)benzenesulfonic acid; 2-[2-[3-[3,5-Bis(trifluoromethyl)phenyl]ureido]-4-trifluoromethylphenoxy]-benzenesulfonic acid; 2-[2-(Benzamido)-4-trifluoromethylphenoxy]-5-(1,1-dimethylpropyl)benzenesulfonic acid; 25 and 2-[2-[3,5-bis(trifluoromethyl)benzamido-4-trifluoromethylphenoxy]-5-(1,1-dimethylpropyl)benzenesulfonic acid.

**Assay (c): Arachidonic Acid Release Assay**

30 **Preparation of human neutrophils**

Human neutrophils are obtained in the laboratory using three different methods. One method uses leukophoresis packs from normal humans and neutrophils are isolated using the histopaque-1077 technique. The blood is centrifuged at 300 x g for 15 minutes. The cell pellets are resuspended in PBS composed of 137 mM NaCl, 8.8 mM Na<sub>2</sub>HPO<sub>4</sub>, 35 1.5 mM KH<sub>2</sub>PO<sub>4</sub>, 2.7 mM KCl (Dulbecco's Gibco Laboratories, Long Island, New York) and layered over histopaque-1077 (Sigma, St. Louis, Missouri). The pellets are collected after centrifugation (300 x g for 30 minutes) and washed once in PBS. The cell pellets are

exposed briefly to deionized water to lyse any erythrocytes. The remaining cells are collected by centrifugation, suspended in PBS, counted and identified after cytopinning and staining. The final leukocyte preparation will be of greater than 95% purity and viability.

5        The second method isolates human neutrophils from fresh heparinized normal blood using the Histopaque-1077 technique. The blood is layered over Histopaque-1077 (Sigma, St. Louis Missouri) and centrifuged at 400 x g for 30 minutes. The cell pellets are resuspended in 35 ml of PBS and 12 ml of 6% Dextran, followed by Dextran sedimentation at room temperature for 45 minutes. The upper layer is collected and further centrifugated  
10      for 10 minutes at 1000 rpm. The cell pellets are exposed briefly to deionized water to lyse erythrocytes. The remaining cells are collected by centrifugation, suspended in PBS, counted and identified after cytopinning and staining. The final leukocyte preparation will be of greater than 95% purity and viability.

15      The third method isolates human neutrophils from freshly drawn heparinized normal blood using the Percoll technique. The blood is first treated with 6% Dextran at room temperature for a 1 hour sedimentation. The upper layers of plasma are collected and centrifuged at 400 x g for 10 minutes. The cell pellets are resuspended in Percoll 1.070 g/ml supplemented with 5% fetal bovine serum and layered on discontinuous gradients (1.080, 1.085, 1.090, 1.095 g/ml) followed by centrifugation at 400 x g for 45 minutes.  
20      The neutrophils are collected from interfaces of 1.080 and 1.085 and the 1.085 and 1.090 Percoll densities, followed by a centrifugation at 400 x g for 45 minutes. The neutrophils are suspended in PBS, counted and identified after cytopinning and staining. The final leukocyte preparation will be of greater than 95% purity and viability.

25      There should be no difference noted in the response of the neutrophils nor in the effects of test compounds in neutrophils isolated by the three different techniques.

#### Treatment of human neutrophils

Neutrophils are suspended in PBS with 1 mM  $\text{Ca}^{2+}$  and 1.1 mM  $\text{Mg}^{2+}$  at concentrations of 5 to 20  $\times 10^6$  cells per ml. Cells are added to test tubes and treated with  
30      the desired compounds for 5 to 10 minutes, then challenged with calcium ionophore A23187, 2  $\mu\text{M}$ , or vehicle control, PBS containing 0.25-1 mg/ml BSA. After 5 to 20 minutes, the reactions are terminated by addition of an equal volume of chloroform:methanol (1:2, v/v) to the samples. [ $^2\text{H}_8$ ]Arachidonic acid (50, 100 or 200 ng) is added as an internal standard and the lipids were extracted by addition of equal volumes of chloroform and  
35      distilled water. The samples are vortexed and centrifuged at high speed and the chloroform layer removed to a clean tube.

Assay for free arachidonic acid

The chloroform extract for each sample is evaporated to dryness and the material resuspended in hexane. The hexane is passed through a Silica solid phase column (500 mg), washed 2x with hexane and a fatty acid enriched fraction eluted with hexane:ethyl ether (1:1, v/v). Solvents are removed from the samples under a stream of nitrogen then the samples are converted to pentafluorobenzyl esters using pentafluorobenzyl bromide and diisopropylethylamine in acetonitrile. Solvents are removed and samples are suspended in hexane. GC/MS analysis is performed on a suitable instrument, such as a Finnigan MAT TSQ 700 GC/MS/MS/DS (San Jose, California) operated as a single stage quadrupole system or a Hewlett-Packard 5890 with a 5989A M5 system.

10 The peaks corresponding to arachidonic acid and [<sup>2</sup>H<sub>8</sub>]Arachidonic acid are identified and the areas of those peaks compared and the released arachidonic acid calculated as ng of arachidonic acid for each sample.

15 Protein concentrations are assessed using the protein assay reagents from Bio-Rad (Richmond, CA).

Assay (d): Assay for Production of Platelet-Activating Factor (PAF)

20

Preparation of human neutrophils:

Blood is obtained from normal humans and neutrophils are isolated as described for the arachidonic acid release assay, above. The final leukocyte preparation should be of greater than 95% purity and viability.

25

Treatment of human neutrophils

Neutrophils are suspended in PBS at concentrations of 5 to 20 x 10<sup>6</sup> cells per ml. Cells are added to test tubes and treated with the desired compounds for 5 to 10 minutes, then challenged with calcium ionophore A23187, 2 µM and 20-30 µCi of [<sup>3</sup>H]acetic acid (NEN-Dupont, Boston, Massachusetts), or the vehicle of PBS with 0.25-1 mg/ml. After 5 to 20 minutes, the reactions are terminated by addition of an equal volume of chloroform:methanol (1:2, v/v) to the samples and the lipids are extracted by addition of equal volumes of chloroform and distilled water. The samples are vortexed and centrifuged at high speed and the chloroform layer removed to a clean tube.

35

Assay for PAF

The chloroform from each tube is evaporated to dryness and the material suspended in a small volume of chloroform or chloroform:methanol (25-100 µl) and the total material spotted on a Silica TLC plate. The plates are developed in chloroform/methanol/ acetic

acid/water (50:25:8:4, v/v) visualized by radioscanning (Bioscan) and the product, [<sup>3</sup>H]PAF, is scraped and quantified by liquid scintillation spectroscopy. With this TLC system, the R<sub>f</sub> value for a synthetic standard of PAF is approximately 0.33.

5

Assay (e): Methods for the evaluation of CoA-IT inhibitors on mobilization of labeled arachidonic acid in intact cells

Measurement of the effect of CoA-IT inhibitors on the transfer of [<sup>3</sup>H]arachidonate into 1-ether phospholipids in non-stimulated inflammatory cells can be accomplished by 10 general application of the following specific method. Human neutrophils are isolated and resuspended (5 x 10<sup>7</sup>/ml) in Hanks Balanced Salt Solution (HBSS; Gibco). [5,6,8,9,11,12,14,15-<sup>3</sup>H]-Arachidonic acid (100 Ci/mmol; New England Nuclear) complexed to 200 $\mu$ l HBSS containing 0.25 mg/ml HSA is added to the cell suspension (1  $\mu$ Ci/ml). The cells are incubated with gentle shaking at 37°C for 5 min. The reaction is 15 terminated by the addition of 40 ml ice-cold HBSS containing HSA (0.25 mg/ml). The cells are then removed from the supernatant fluid by centrifugation (225 g, 8 min). Unincorporated [<sup>3</sup>H]-arachidonic acid is completely removed by two more washes of HBSS containing 0.25 mg/ml HSA. The neutrophils are resuspended in fresh buffer, exposed to various concentrations of a CoA-IT inhibitor or its vehicle and incubated without stimulation 20 for 2 hrs. At that time, the tubes containing the cells and buffer are extracted (Bligh & Dyer [Can. J. Biochem. Physiol. (1959) 37, 911-917]) and the phospholipid classes are separated and collected by normal phase HPLC, using a Ultrasphere Silica column (4.6 mm x 250 mm; Rainin) eluted with hexane/2-propanol/ethanol/phosphate buffer (pH 7.4)/acetic acid (490:367:100:30:0.6 v/v) for 5 min at a flow rate of 1 ml/min. The amount of 25 phosphate buffer in the eluting solvent is increased to 5 % over 10 min and this solvent composition is maintained until all the phospholipid classes is eluted from the column (30-40 min) (Chilton, F. H. [Methods Enzymol. (1990) 187, 157-166]). The phospholipids are converted into diradylglycerols by addition of phospholipase C, 20 units-40 units of *Bacillus cereus* phospholipase C (Sigma Type XIII) in 100 mM Tris HCl buffer (pH 7.4) 30 for 2.5-6 hr, then converted into 1,2-diradyl-3-acetylglycerols by incubation with acetic anhydride and pyridine (Chilton, F. H. [Methods Enzymol. (1990) 187, 157-166]). The phospholipid subclasses are separated by TLC in benzene/hexane/ethyl ether (50:45:4, v/v), located by image analysis (Bioscan) and the amount of radioactivity in each class is determined by zonal scraping and liquid scintillation counting.

35

The following is the method for assessing the ability of a compound to alter arachidonate content of cellular phospholipids, which can be generalized for any desired

cell. Specifically, mouse bone marrow-derived mast cells are removed from culture and provided with exogenous [<sup>3</sup>H]arachidonic acid for 30 minutes. The labeled arachidonic acid which had not been incorporated into the cells is then removed by washing the cells 2 times with an albumin-containing buffer. At that point, the cells are treated with various 5 concentrations of CoA-IT inhibitors and then placed back in culture for 24-48 hours. The phospholipids are extracted by the method of Bligh and Dyer [Can. J. Biochem. Physiol. (1959) 37, 911-917] and phospholipids separated by normal phase HPLC by the method of Chilton [Methods Enzymol. (1990)187, 157-166]. The radioactive and mole quantities of arachidonate in complex lipids are determined. At this point, cellular lipid extracts are treated 10 with KOH (0.5 M) to remove fatty acids from complex lipids (phospholipids) and the quantities of arachidonate in these extracts can then be determined by various methods, including gas chromatography and mass spectrometry (Chilton [Methods Enzymol. (1990)187, 157-166]).

15

Assay (f) for measurement of stimulated eicosanoid release by human monocytes.

Human Monocyte Isolation. Leukocyte-rich leukopaks obtained from Biological Specialties (Lansdale, PA) are collected from male volunteers who are not taking anti-inflammatoary drugs. Leukopaks are centrifuged (90 x g for 15 min) twice to remove the platelet-rich plasma. The cell pellet is washed by centrifugation and are resuspended in HBSS without Ca<sup>2+</sup> or Mg<sup>2+</sup>. Histopaque 1077 is layered under the cell suspension and centrifuged at 400 x g for 30 min to obtain the buffy coat. The interfacial buffy coat, containing monocytes and lymphocytes, is removed and saved. The buffy coat is washed 20 twice with HBSS without Ca<sup>2+</sup> or Mg<sup>2+</sup> by centrifugation. The cell pellet (4-6 x 10<sup>8</sup> cells/30mls) is resuspended in iso-osmotic media (RPMI-1640, 10% heat inactivated fetal bovine serum (FBS), 0.2 mM L-glutamine, 2.5 mM HEPES) and layered over an equal volume of 46% Percoll mixture (10 X PBS/ Percoll; 9.25 / 0.75) and 54% iso-osmotic media and centrifuged for 30 min at 1000 x g (Marshall and Roshak, Biochem. Cell Biol. 71: 331- 25 339, 1993). The monocyte population located at the interface of the Percoll gradient is removed and washed twice in HBSS without Ca<sup>2+</sup> or Mg<sup>2+</sup>. This resulted in a greater than 30 85-90 % pure monocyte population as assessed by differential staining.

Measurement of Stimuli-Induced Eicosanoid Release. Monocytes (5 x 10<sup>6</sup>/ml) are 35 incubated as a suspension in serum-free RPMI-1640 medium containing the vehicle DMSO (< 1%) or drug for 30 min at 27°C after which vehicle or stimuli is added for the indicated time. The stimulating agent is solubilized in DMSO and appropriate vehicle controls are

included in all experiments. The amount of stimuli is chosen from the linear portion of a concentration versus product curve usually representing 60-80% maximal stimulation over the indicated incubation time at 37°C (A23187, 1 µM, (15 min). The reaction is terminated by reduction of pH through addition of citric acid and centrifugation (10 min, 400 x g, 4°C).

5 Cell viability is monitored before and after experiments using trypan blue exclusion. The cell-free media is decanted and stored at -70° C until analyzed. Prostaglandin E<sub>2</sub> and LTC<sub>4</sub> are directly measured in cell-free media using enzyme immunoassay (EIA) kits purchased from Caymen Chemical Co. (Ann Arbor, MI). Sample or standard dilutions are made with appropriate media and analyzed in triplicate. Results are obtained by extrapolation from a

10 standard curve prepared in the media and expressed as pg or ng/ml of sample.

*In vivo assays*

15 Assays (g and h) : Assay (Method) for TPA (assay g) or Arachidonic acid (assay h)-induced Inflammation

Animals:  
Male Balb/c inbred mice are obtained from Charle River Breeding Laboratories

20 (Kingston, NY). Within a single experiment mice (22-25g) are age-matched. These *in vivo* experiments typically involve the use of 5-6 animals/group.

(g) TPA-induced Mouse Ear Inflammation:

Assay of Ear Edema

25 TPA (12-O-tetradecanoylphorbol 13-acetate) (Sigma Chemical Company) in acetone (4 mg/20ml) is applied to the inner and outer surfaces of the left ear of BALB/c male mice. The thickness of both ears is then measured with a dial micrometer (Mitutoyo, Japan) at both 2 and 4 hours after treatment, and the data expressed as the change in thickness (10<sup>-3</sup>cm) between treated and untreated ears. The application of acetone does not cause an

30 edematous response; therefore, the difference in ear thickness represents the response to the TPA. After measuring the edema, the inflamed left ears are removed and stored at -70°C until they were assayed for MPO (myeloperoxidase) activity where appropriate.

Assay of myeloperoxidase (MPO) in Inflamed Ear Tissue:

35 On the day of the assay, partially thawed ear tissues are minced and then homogenized (10% w/v) with a Tissumizer homogenizer (Tekmar Co.) in 50 mM phosphate buffer (pH 6) containing 0.5% HTAB. The tissue homogenates are taken through three cycles of freeze-thaw, followed by brief sonication (10 sec). The method of Bradley et al.

is used with modifications as described. The appearance of a colored product from the MPO-dependent reaction of o-dianisidine (0.167 mg/ml; Sigma) and hydrogen peroxide (0.0005%; Sigma) is measured spectrophotometrically at 460 nm. Supernatant MPO activity is quantified kinetically (change in absorbance measured over 3 min, sampled at 15-  
5 sec intervals) using a Beckman DU-7 spectrophotometer and a Kinetics Analysis package (Beckman Instruments, Inc.). One unit of MPO activity is defined as that degrading one micromole of peroxide per minute at 25°C.

Statistics:

10 Statistical analysis is done using Student's "t" test. The ED<sub>50</sub> are values which cause a 50% inhibition of the inflammatory response and are calculated by regression analysis of the dose response data.

(h) Arachidonic acid induced ear inflammation assay

15 Arachidonic acid is dissolved in acetone (1mg/ear) to the left ear of BALB/c male mice. The thickness of both ears is measured with a constant pressure thickness guage 1 hour after treatment and the data expressed as the change in thickness between treated and untreated ears. Test compounds or vehicle are given at the time of AA application. The inflammatory cell infiltration is measured by MPO activity as described above in the TPA ear  
20 edema assay. After the edema measurements are made, the inflamed ears are removed and assayed for MPO activity.

The anti-inflammatory effect of various standard inhibitors topically administered in the AA and TPA induced mouse ear edema model were measured for dexamethasone, 25 scalaradial and Wyeth's compound WY 50,295 at doses of 0.2, 0.1 and 0.3 respectively. The TPA % change in edema was -50 (p<0.001), -46 (p<0.01) and -18 (ns) respectively; for AA the change was -10 (ns), -11(ns) and -50 (p<0.001). The change in MPO for TPA model was -54 (p<0.001), -65 (p<0.001) and -36 (p<0.05) respectively; for AA it was 0 (ns), -33 (ns) and -90 (p<0.001). One hypothesis is that the AA administration to the ear 30 overrides the need for PLA<sub>2</sub> mediated liberation of substrate for subsequent pro-inflammatory lipid mediator generation or AA mobilization by CoA-IT. In this case an inhibitor of an AA-metabolizing enzyme should be effective while an inhibitor of PLA<sub>2</sub> would be ineffective. As noted above, scalaradial and dexamethasone have little or no effect in the AA ear model at concentrations which were effective in the TPA ear model. This can 35 be contrasted to the activity of the selective 5-LO inhibitor WY 50,295 which strongly inhibits inflammation in response to AA. The AA ear model therefore responds well to compounds that exhibit 5-LO inhibitory action and appears to be unaffected by putative

PLA<sub>2</sub> inhibitors. This model therefore provides a unique tool with which the contribution of the 5-LO inhibition to the *in vivo* anti-inflammatory activity of various compounds can be separated from LMW-PLA<sub>2</sub> inhibition.

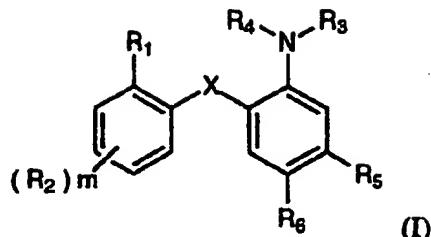
5 As used herein, various abbreviations and explanations are as follows: [<sup>3</sup>H], a molecule that contains tritium atoms, a radioactive isotope; A23187, a compound that allows free entry of calcium into a cell; AA, arachidonic acid; arachidonate, arachidonic acid contained within a phospholipid; free arachidonic acid, arachidonic acid that is not contained within a phospholipid; [<sup>2</sup>H<sub>8</sub>]arachidonic acid, the form of arachidonic acid labeled with 8 deuterium atoms, a stable isotope; 1-alkyl, 1-O-alkyl; 1-alkenyl, 1-O-alk-1'-enyl; BSA, bovine serum albumin; CoA, coenzyme A; CoA-IT, CoA-independent transacylase; DTT, dithiothreitol; EGTA, [ethylenebis(oxyethylenenitrilo)]tetra acetic acid, a calcium chelator; GPC, sn-glycero-3-phosphocholine; EDTA, a metal ion chelator; GPE, sn-glycero-3-phosphoethanolamine; GC/MS, gas chromatography and mass spectrometry; 5HETE, 5(S)-hydroxyeicosa-6,8,11,14-tetraenoic acid; 15HETE, 15(S)-hydroxyeicosa-5,8,11,13-tetraenoic acid; HL-60, American Type Tissue Culture designated cell line similar to a monocyte; LTB<sub>4</sub>, leukotriene B<sub>4</sub>; LTC<sub>4</sub>, leukotriene C<sub>4</sub>; LTD<sub>4</sub>, leukotriene D<sub>4</sub>; lyso PAF, 1-alkyl-2-lyso-GPC, lyso platelet-activating factor; PLA<sub>2</sub>, phospholipase A<sub>2</sub>; PBS, phosphate buffered saline; PAF, platelet activating factor, 1-alkyl-2-acetyl-GPC; PL, 20 phospholipid; PC, phosphatidylcholine; PE, phosphatidylethanolamine, PI, phosphatidylinositol; PMN, polymorphonuclear neutrophilic cell, neutrophil; PS phosphatidylserine; Rf, the distance a compound travels as a fraction of the solvent front; TLC, thin layer chromatography; U937, American Type Tissue Culture designated cell line similar to a monocyte.

25

The above description fully discloses the invention including preferred embodiments thereof. Modifications and improvements of the embodiments specifically disclosed herein are within the scope of the following claims. Without further elaboration, it is believed that one skilled in the art can, using the preceding description, utilize the present invention to its 30 fullest extent. Therefore, the Examples herein are to be construed as merely illustrative and not a limitation of the scope of the present invention in any way. The embodiments of the invention in which an exclusive property or privilege is claimed are defined as follows.

What is claimed is:

1. A compound of the formula :



5

wherein

R<sub>1</sub> is SO<sub>3</sub>H or S(O)<sub>n</sub>C<sub>1-4</sub> alkyl;

n is 0 or an integer having a value of 1 or 2;

10 R<sub>2</sub> is hydrogen, halogen, optionally substituted C<sub>1-8</sub> alkyl, or C<sub>1-8</sub> alkoxy;

R<sub>3</sub> is -C(O)R<sub>7</sub> or -C(S)R<sub>7</sub>;

R<sub>4</sub> is hydrogen, or C<sub>1-4</sub> alkyl;

R<sub>5</sub> is hydrogen, halogen, CF<sub>3</sub>, CH<sub>3</sub>, (CH<sub>2</sub>)<sub>m</sub>C(O)R<sub>8</sub>, or (CH<sub>2</sub>)<sub>m</sub>OH;

m is 0 or an integer having a value of 1 or 2;

15 R<sub>6</sub> is hydrogen or halogen;

R<sub>7</sub> is NR<sub>9</sub>R<sub>10</sub>;

R<sub>8</sub> is hydrogen or C<sub>1-4</sub> alkyl;

R<sub>9</sub> is optionally substituted aryl, optionally substituted arylC<sub>1-2</sub> alkyl, optionally substituted C<sub>1-8</sub> alkyl, or R<sub>9</sub> and R<sub>10</sub> together with the nitrogen to which they are

20 attached form a 5 to 7 membered saturated or unsaturated ring which may optionally comprise an additional heteroatom selected from O/N or sulfur;

X is oxygen or sulfur;

provided that

25 a) when R<sub>2</sub> is 2,2-dimethyl propyl, m is 1, R<sub>1</sub> is SO<sub>3</sub>H, R<sub>5</sub> is CF<sub>3</sub>, and R<sub>3</sub> is C(O)NR<sub>9</sub>R<sub>10</sub>, and R<sub>9</sub> is hydrogen, than R<sub>10</sub> is other than 3,5-trimethyl phenyl ; or

b) when R<sub>2</sub> is 2,2-dimethyl propyl, m is 1, R<sub>1</sub> is SO<sub>3</sub>H, R<sub>5</sub> is CF<sub>3</sub>, and R<sub>3</sub> is C(O)NR<sub>9</sub>R<sub>10</sub> and R<sub>9</sub> is hydrogen, than R<sub>10</sub> is other than 4-chloro-3-trifluoromethyl phenyl ;

30 c) when R<sub>2</sub> is 2,2-dimethyl propyl, m is 1, R<sub>1</sub> is SO<sub>3</sub>H, R<sub>5</sub> is CF<sub>3</sub>, and R<sub>3</sub> is C(O)NR<sub>9</sub>R<sub>10</sub> and R<sub>9</sub> is hydrogen, than R<sub>10</sub> is other than 3-trifluoromethyl phenyl ;

d) when R<sub>1</sub> is SO<sub>3</sub>H, R<sub>2</sub> is 5-methyl-4-chloro, m is 2, R<sub>5</sub> is chlorine, R<sub>6</sub> is hydrogen, and R<sub>3</sub> is C(O)NR<sub>9</sub>R<sub>10</sub> and R<sub>9</sub> is hydrogen, than R<sub>10</sub> is other than a 3-CF<sub>3</sub>-4-

chlorophenyl, 3,4-dichlorophenyl, or 2-methyl-6-chlorophenyl;

5 e) when R<sub>1</sub> is SO<sub>3</sub>H, R<sub>2</sub> is 4,6-dichlorine, m is 2, R<sub>5</sub> is chlorine, R<sub>6</sub> is hydrogen, and R<sub>3</sub> is C(O)NR<sub>9</sub>R<sub>10</sub> and R<sub>9</sub> is hydrogen, than R<sub>10</sub> is other than a 2-methyl-4-chlorophenyl;

10 f) when R<sub>1</sub> is SO<sub>3</sub>H, R<sub>2</sub> is 4-chloro, m is 1, R<sub>5</sub> is chlorine, R<sub>6</sub> is hydrogen, and R<sub>3</sub> is C(O)NR<sub>9</sub>R<sub>10</sub> and R<sub>9</sub> is hydrogen, than R<sub>10</sub> is other than a 2,5-dichlorophenyl, 3,5-di-trifluoromethyl phenyl, 2-chloro-5-CF<sub>3</sub>-phenyl, 3-CF<sub>3</sub>-4-chlorophenyl, 3,4-dichlorophenyl, 2,3-dichlorophenyl, 4-chlorophenyl, 3-trifluoromethylphenyl, 3-chloro-4-(4-chlorophenoxy), 3-CF<sub>3</sub>-6-(4-chlorophenoxy), 3-chloro-6-(4-chlorophenoxy);

15 g) when R<sub>1</sub> is SO<sub>3</sub>H, R<sub>2</sub> is 4,5-dichloro, m is 2, R<sub>5</sub> is chlorine, R<sub>6</sub> is hydrogen, and R<sub>3</sub> is C(O)NR<sub>9</sub>R<sub>10</sub> and R<sub>9</sub> is hydrogen, than R<sub>10</sub> is other than a 2-chloro-5-CF<sub>3</sub>-phenyl or 3-CF<sub>3</sub>-4-chlorophenyl;

20 h) when R<sub>1</sub> is SO<sub>3</sub>H, R<sub>2</sub> is 4,5-dichloro, m is 2, R<sub>5</sub> is CF<sub>3</sub>, R<sub>6</sub> is hydrogen, and R<sub>3</sub> is C(O)NR<sub>9</sub>R<sub>10</sub> and R<sub>9</sub> is hydrogen, than R<sub>10</sub> is other than a 4-chloro-3-CF<sub>3</sub>phenyl;

i) when R<sub>1</sub> is SO<sub>3</sub>H, X is sulfur, R<sub>2</sub> is 4,5-dichloro, m is 2, R<sub>5</sub> is trifluoromethyl, R<sub>6</sub> is hydrogen, and R<sub>3</sub> is C(O)NR<sub>9</sub>R<sub>10</sub> and R<sub>9</sub> is hydrogen, than R<sub>10</sub> is other than a phenyl substituted one to two times independently with chlorine or trifluoromethyl;

j) when R<sub>1</sub> is SO<sub>3</sub>H, X is sulfur, R<sub>2</sub> is 4-chloro, m is 1, R<sub>5</sub> is trifluoromethyl, R<sub>6</sub> is hydrogen, and R<sub>3</sub> is C(O)NR<sub>9</sub>R<sub>10</sub> and R<sub>9</sub> is hydrogen, than R<sub>10</sub> is other than a phenyl substituted one to two times independently with chlorine or trifluoromethyl;

25 k) when R<sub>1</sub> is SO<sub>3</sub>H, X is sulfur, R<sub>2</sub> is 4-chloro, m is 1, R<sub>5</sub> is chlorine, R<sub>6</sub> is hydrogen, and R<sub>3</sub> is C(O)NR<sub>9</sub>R<sub>10</sub> and R<sub>9</sub> is hydrogen, than R<sub>10</sub> is other than a phenyl substituted one to two times independently with chlorine or trifluoromethyl;

l) or a pharmaceutically acceptable salt thereof.

2. The compound according to Claim 1 wherein R<sub>7</sub> is NR<sub>9</sub>R<sub>10</sub>.

3. The compound according to Claim 2 wherein R<sub>9</sub> is hydrogen, and R<sub>10</sub> is hydrogen, and R<sub>3</sub> is an optionally substituted aryl, an optionally substituted arylC<sub>1-2</sub> alkyl, or optionally substituted C<sub>1-8</sub> alkyl.

4. The compound according to Claim 1 wherein X is oxygen.

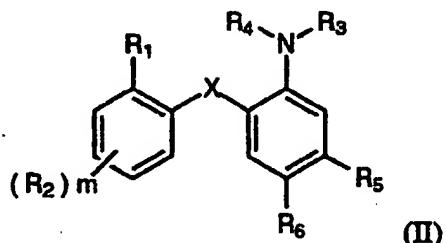
35 5. The compound according to Claim 1 wherein R<sub>2</sub> is hydrogen, halogen, or optionally substituted C<sub>1-8</sub> alkyl.

6. The compound according to Claim 1 wherein R<sub>4</sub> is hydrogen.
7. The compound according to Claim 1 wherein R<sub>5</sub> is hydrogen, halogen, or CF<sub>3</sub>.
- 5 8. The compound according to Claim 1 which is  
2-[2-(3-Phenylureido)-4-trifluoromethylphenoxy]-5-(1,1-dimethylpropyl)benzenesulfonic acid;  
2-[2-(3-Phenylureido)phenoxy]-5-(1,1-dimethylpropyl)benzenesulfonic acid; 2-[2-[3-[3,5-Bis(trifluoromethyl)phenyl]ureido]phenoxy]-5-(1,1-dimethylpropyl)benzenesulfonic acid;
- 10 10 2-[2-[3-(4-Phenoxyphenyl)ureido]-4-trifluoromethylphenoxy]-5-(1,1-dimethylpropyl)-benzenesulfonic acid;  
2-[2-[3-(4-Methoxyphenyl)ureido]-4-trifluoromethylphenoxy]-5-(1,1-dimethylpropyl)-benzenesulfonic acid;  
2-[2-[3-[3,5-Bis(trifluoromethyl)phenyl]ureido]-4-trifluoromethylphenoxy]-5-(1,1,3,3-tetramethylbutyl)benzenesulfonic acid;
- 15 15 2-[2-[3-[3,5-bis(Trifluoromethyl)phenyl]ureido]-4-methylphenoxy]-5-(1,1-dimethylpropyl)benzenesulfonic acid;  
2-[2-[3-[3,5-bis(trifluoromethyl)phenyl]thioureido]-4-(trifluoromethyl)phenoxy]-5-(1,1-dimethylpropyl)benzenesulfonic acid; and
- 20 20 2-[2-[3-[3,5-Bis(trifluoromethyl)phenyl]ureido]-4-trifluoromethylphenoxy]-benzenesulfonic acid.
9. A pharmaceutical composition comprising a pharmaceutically acceptable diluent or carrier and a compound according to Claim 1.
- 25 10. A method for treating an inflammatory disease or disorder in a mammal in need thereof which method comprises administering to said mammal an effective amount of a compound according to Claim 1.
- 30 11. The method according to Claim 10 wherein the inflammatory disease or disorder is allergic rhinitis, ischemia, reperfusion injury, arthritis, inflammatory bowel disease, Crohn's disease, ulcerative colitis, asthma, adult respiratory distress syndrome, anaphylaxis, actinic keratosis, psoriasis, contact dermatitis, or pyresis.
- 35 12. The method according to Claim 11 wherein the inflammatory disease or disorder is mediated by lipid inflammatory mediators, arachidonic acid, its metabolites and/or platelet activating factor (PAF).

13. The method according to Claim 12 wherein the lipid inflammatory mediators are inhibited by the an inhibitor of the enzyme phospholipase A2 (PLA2) or Coenzyme A independent transacylase (CoA-IT).

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14. A compound of the formula:



wherein

10 R<sub>1</sub> is SO<sub>3</sub>H or S(O)<sub>n</sub>C<sub>1-4</sub> alkyl;

n is 0 or an integer having a value of 1 or 2;

R<sub>2</sub> is hydrogen, halogen, optionally substituted C<sub>1-8</sub> alkyl, or C<sub>1-8</sub> alkoxy;

n is an integer having a value of 1 or 2;

R<sub>3</sub> is -C(O)R<sub>7</sub> or -C(S)R<sub>7</sub>;

15 R<sub>4</sub> is hydrogen, or C<sub>1-4</sub> alkyl;

R<sub>5</sub> is hydrogen, halogen, CF<sub>3</sub>, CH<sub>3</sub>, (CH<sub>2</sub>)<sub>t</sub>C(O)R<sub>8</sub>, or (CH<sub>2</sub>)<sub>t</sub>OH;

t is 0 or an integer having a value of 1 or 2;

R<sub>6</sub> is hydrogen or halogen;

R<sub>7</sub> is optionally substituted aryl, optionally substituted arylC<sub>1-2</sub> alkyl, or optionally

20 substituted C<sub>1-8</sub> alkyl;

R<sub>8</sub> is hydrogen or C<sub>1-4</sub> alkyl;

X is oxygen or sulfur;

or a pharmaceutically acceptable salt thereof.

25 15. The compound according to Claim 14 wherein R<sub>7</sub> is an optionally substituted aryl or an optionally substituted aryl alkyl.

16. The compound according to Claim 15 wherein the aryl moiety or aryl alkyl moiety is a phenyl or benzyl which group is optionally substituted one to two times by halogen or

30 trifluoromethyl.

17. The compound according to Claim 14 wherein X is oxygen.

18. The compound according to Claim 14 wherein R<sub>2</sub> is hydrogen, halogen, or optionally substituted C<sub>1-8</sub> alkyl.
- 5 19. The compound according to Claim 14 wherein R<sub>4</sub> is hydrogen.
20. The compound according to Claim 14 wherein R<sub>5</sub> is hydrogen, halogen, or CF<sub>3</sub>, 2-[2-(Phenylmethylacetamido)phenoxy]-5-(1,1-dimethylpropyl)benzenesulfonic acid; 2-[2-(Benzamido)-4-trifluoromethylphenoxy]-5-(1,1-dimethylpropyl)benzenesulfonic acid;
- 10 2-[2-[3,5-bis(trifluoromethyl)benzamido-4-trifluoromethylphenoxy]-5-(1,1-dimethylpropyl)benzenesulfonic acid;
21. A pharmaceutical composition comprising a pharmaceutically acceptable diluent or carrier and a compound according to Claim 14.
- 15 22. A method for treating an inflammatory disease or disorder in a mammal in need thereof which method comprises administering to said mammal an effective amount of a compound according to Claim 14.
- 20 23. The method according to Claim 22 wherein the inflammatory disease or disorder is allergic rhinitis, ischemia, reperfusion injury, arthritis, inflammatory bowel disease, Crohn's disease, ulcerative colitis, asthma, adult respiratory distress syndrome, analphylaxis, actinic keratosis, psoriasis, contact dermatitis, or pyresis.
- 25 24. The method according to Claim 23 wherein the inflammatory disease or disorder is mediated by lipid inflammatory mediators, arachidonic acid, its metabolites and/or platelet activating factor (PAF).
- 30 25. The method according to Claim 24 wherein the lipid inflammatory mediators are inhibited by the an inhibitor of the enzyme phospholipase A<sub>2</sub> (PLA<sub>2</sub>) or Coenzyme A independent transacylase (CoA-IT).

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US95/06961

## A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :Please See Extra Sheet.

US CL :Please See Extra Sheet.

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : Please See Extra Sheet.

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US, A, 2,715,643 (MARTIN) 16 August 1955. Entire document.	1-9
X	US, A, 2,649,476 (MARTIN) 18 August 1953. Entire document.	1-9
X	US, A, 2,311,062 (MARTIN ET AL) 16 February 1943. Entire document.	1-9

 Further documents are listed in the continuation of Box C. See patent family annex.

• Special categories of cited documents:	
• "A" document defining the general state of the art which is not considered to be of particular relevance	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
• "E" earlier document published on or after the international filing date	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
• "L" document which may throw doubt on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
• "O" document referring to an oral disclosure, use, exhibition or other means	"Z" document member of the same patent family
• "P" document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

22 AUGUST 1995

Date of mailing of the international search report

07 SEP 1995

Name and mailing address of the ISA/US  
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**INTERNATIONAL SEARCH REPORT**

International application No.

PCT/US95/06961

**Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)**

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1.  **Claims Nos.:**  
because they relate to subject matter not required to be searched by this Authority, namely:
  
2.  **Claims Nos.:**  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
  
3.  **Claims Nos.:**  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

**Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

- I. Claims 1-13, drawn to urea and thiourea derivatives.
- II. Claims 14-25, drawn to amido and thioamido derivatives.

The inventions listed as groups I and II do not relate to a single inventive concept under PCT Rule 13.1, because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

The two groups of inventions involve different chemical functional entities that do not behave similarly in chemical and biological interactions.

1.  As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2.  As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3.  As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
  
4.  No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: 1-13

**Remark on Protest**

The additional search fees were accompanied by the applicant's protest.  
 No protest accompanied the payment of additional search fees.

**INTERNATIONAL SEARCH REPORT**

International application No.

PCT/US95/06961

**A. CLASSIFICATION OF SUBJECT MATTER:**

IPC (6):

**A61K 31/17, 31/185, 31/19, 31/24;**  
**C07C 275/28, 275/34, 275/40, 309/32.**

**A. CLASSIFICATION OF SUBJECT MATTER:**

US CL :

**514/598, 596, 587, 585, 576, 564, 562, 539;**  
**560/13, 34;**  
**562/47, 48, 430, 439;**  
**564/26, 29, 49, 52.**

**B. FIELDS SEARCHED**

Minimum documentation searched

Classification System: U.S.

**514/598, 596, 587, 585, 576, 564, 562, 539;**  
**560/13, 34;**  
**562/47, 48, 430, 439;**  
**564/26, 29, 49, 52.**